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(71) Applicant *(for all designated States except US)*: **NEURO-GENETICS, INC.** [US/US]; 11085 North Torrey Pines Road, Suite 300, La Jolla, CA 92037 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KOUNNAS, Maria** [US/US]; 3752 Vista de la Bahia, San Diego, CA 92117 (US). **PATRICK, Aaron** [US/US]; 1335 Monroe Street, #304, Denver, CO 80206 (US). **VELICELEBI, Gonul** [US/US]; 4688 Tarantella Lane, San Diego, CA 92130 (US). **WAGNER, Steven** [US/US]; 2315 Wilbur Avenue, San Diego, CA 92109 (US).

(74) Agents: **SEIDMAN, Stephanie, L.** et al.; Heller Ehrman White & McAuliffe LLP, 4350 La Jolla Village Drive. 7th Floor, San Diego, CA 92122-1246 (US).

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(54) Title: METHODS AND COMPOSITIONS FOR MODULATING AMYLOID BETA

(57) Abstract: Provided herein are methods and compositions for detecting, assessing and modulating β-amyloid peptide (Aβ) levels and/or processing of amyloid precursor protein. Methods for screening and/or identifying agents that modulate processing of APP or the levels of β-amyloid peptides, and methods for assessing presenilin activity and for modulating lipoprotein receptor-related protein (LRP), are also provided.

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METHODS AND COMPOSITIONS FOR MODULATING AMYLOID BETA

RELATED APPLICATIONS

Benefit of priority under §119(e) is claimed to U.S. Provisional Application Serial No. 60/405,417, filed August 20, 2002, entitled "Methods of Modulating and Identifying Agents that Modulate Processing of Amyloid Precursor Protein" and U.S. Provisional Application Serial No. 60/411,974, filed September 18, 2002, entitled "Methods of Modulating and Identifying Agents that Modulate Processing of Amyloid Precursor Protein." The subject matter and contents, including sequence listings, of each of these provisional applications is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

The field of invention relates to methods and compositions for detecting, assessing and modulating β-amyloid peptide (Aβ) levels and processing of amyloid precursor protein.

BACKGROUND

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the predominant cause of dementia in people over 65 years of age. It is estimated to affect 4 million Americans. Clinical symptoms of the disease begin with subtle short term memory problems. As the disease progresses, difficulty with memory, language and orientation worsen to the point of interfering with the ability of the person to function independently. Other symptoms, which are variable, include myoclonus and seizures. Duration of AD from the first symptoms of memory loss until death is 10 years on average.

The AD brain is characterized by two distinct pathologies; 1) neurofibrillary tangles (NFT), comprised mostly of tau and 2) amyloid plaques, comprised primarily of highly hydrophobic amyloid precursor protein peptides called Aβ peptides. The characteristic Alzheimer's NFTs contain abnormal filaments bundled together in neurons and occupying much of the perinuclear cytoplasm. These filaments contain the microtubule-associated protein tau in a hyperphosphorylated form. "Ghost" NFTs are also observed in AD brains, which presumably mark the location of dead neurons. Aβ

aggregates into antiparallel filaments in a β-pleated sheet structure resulting in the birefringent nature of the AD amyloid. Other neuropathological features include granulovascular changes, neuronal loss, gliosis and the variable presence of Lewy bodies.

Although Aβ is the major component of AD amyloid, other proteins have also been found associated with amyloid plaques, *e.g.*, alpha-1-anti-chymotrypsin (Abraham *et al.* (1988) *Cell 52*:487-501), cathepsin D (Cataldo (1990) *et al. Brain Res. 513*:181-192), non-amyloid component protein (Ueda *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*:11282-11286), apolipoprotein E (apoE) (Namba *et al.* (1991) *Brain Res. 541*:163-166; Wisniewski and Frangione (1992) *Neurosci. Lett. 135*:235-238; Strittmatter *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*:1977-1981), apolipoprotein J (Choi-Mura (1992) *et al. Acta Neuropathol. 83*:260-264; McGeer (1992) *et al. Brain Res. 579*:337-341), heat shock protein 70 (Hamos *et al.* (1991) *Neurology 41*:345-350), complement components (McGeer and Rogers (1992) *Neurology 43*:447-449), alpha2-macroglobin (Strauss *et al.* (1992) *Lab. Invest. 66*:223-230), interleukin-6 (Strauss *et al.* (1992) *Lab. Invest. 66*:223-230), proteoglycans (Snow *et al.* (1987) *Lab. Invest. 58*:454-458), and serum amyloid P (Coria *et al.* (1988) *Lab. Invest. 58*:454-458).

Plaques are often surrounded by astrocytes and activated microglial cells expressing immune-related proteins, such as the MHC class II glycoproteins HLA-DR, HLA-DP and HLA-DQ, as well as MHC class I glycoproteins, interleukin-2 (IL-2) receptors and IL-1. Also surrounding many plaques are dystrophic neurites, which are nerve endings containing abnormal filamentous structures.Currently, there is no cure or effective treatment for AD and the few approved drugs including Aricept, Exelon, Cognex and Reminyl are palliative at best. Effective treatments are needed. Therefore, among the objects herein, it is an object to provide methods for modulating and for identifying agents for modulating the processing of amyloid precursor protein (APP) and the levels of Aβ peptides. It is also an object to provide methods for identifying candidate agents for the treatment of AD and other neurodegenerative disorders characterized by altered levels of Aβ peptides and/or amyloidosis.

SUMMARY

Provided herein are methods for assessing presenilin activity, comprising contacting a sample containing a presenilin and/or fragment(s) thereof with a lipoprotein receptor-related protein (LRP) and/or fragment(s) thereof; and assessing the processing and/or cleavage of the LRP or fragment(s) thereof. Also provided herein are methods for identifying an agent that modulates presenilin activity, comprising contacting a sample containing a presenilin, and/or fragment(s) thereof, and a lipoprotein receptor-related protein (LRP), and/or fragment(s) thereof with a test agent; and identifying an agent that alters the processing and/or cleavage of LRP and/or fragment(s) thereof. The processing and/or cleavage of LRP and/or fragment(s) thereof can be assessed by determining the presence, absence and/or level of one or more fragments of LRP and/or the composition of LRP. In one embodiment, the step of identifying comprises comparing the cleavage and/or processing of LRP and/or fragment(s) thereof in a test sample that has been contacted with the test agent and a control sample that has not been contacted with the test agent and identifying an agent as an agent that alters the processing and/or cleavage of LRP and/or fragment(s) thereof if the processing and/or cleavage of LRP and/or fragment(s) thereof differs in the test and control samples. The control sample can be the test sample in the absence of test agent. In certain embodiments, the processing or cleavage of LRP and/or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that has a molecular weight of about 20 kD. The LRP fragment that has a molecular weight of about 20 kD can contain an amino acid sequence that is contained within a transmembrane region of LRP. In another embodiment, the LRP fragment that has a molecular weight of about 20 kD can bind with an antibody generated against a C-terminal amino acid sequence of an LRP. The C-terminal amino acid sequence of LRP can be a sequence of about the C-terminal 13 amino acids of an LRP. In another embodiment, the LRP fragment that has a molecular weight of about 20 kD comprises an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10. The LRP fragment that has a molecular weight of about 20 kD can be present when an LRP is not cleaved by a presenilin-dependent activity; or can be in the presence of an

inhibitor of a presenilin-dependent activity. In a particular embodiment, the inhibitor is DAPT.

In particular embodiments of the methods provided herein, the processing and/or cleavage of LRP or fragment(s) thereof can be assessed by determining the presence or absence and/or level of an LRP C-terminal fragment (CTF). The processing and/or cleavage of LRP and/or fragment(s) thereof is assessed by determining the presence or absence and/or level of a fragment of LRP that binds to an antibody. The antibody can bind to an epitope in the C-terminal about 13 amino acids of an LRP, and can be a polyclonal antibody. The sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample. In particular embodiments, the cell can contain presenilin, LRP and/or fragment(s) of presenilin and/or LRP. The cell can be either eukaryotic, mammalian, rodent or a human cell.

Also provided herein are methods for identifying a candidate agent for treatment or prophylaxis of a disease associated with an altered presenilin, comprising contacting a sample that contains an altered presenilin and/or fragment(s) thereof and a lipoprotein receptor-related protein (LRP) and/or fragment(s) thereof with a test agent, wherein the altered presenilin and/or fragment(s) thereof is associated with an altered cleavage and/or processing of LRP and/or fragment(s) thereof; and identifying a candidate agent that restores LRP cleavage and/or processing to substantially that which occurs in the presence of a presenilin and/or fragment(s) thereof that is not associated with an altered cleavage and/or processing of LRP and/or fragment(s) thereof. The presenilin and/or fragment(s) thereof can comprise a mutation, and can be altered relative to a wild-type presenilin, wherein the wild-type is a predominant allele. The wild-type presenilin can be one that occurs in an organism that exhibits normal presenilin-dependent LRP processing patterns. The disease can be an amyloidosis-associated disease; a neurodegenerative disease, such as Alzheimer's Disease. The mutation can be linked to familial Alzheimer's disease.

In one embodiment of the methods provided herein, LRP cleavage and/or

processing is assessed by determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP. The step of identifying can comprise comparing the cleavage and/or processing of LRP and/or fragment(s) thereof in a test sample that has been contacted with the test agent and a control sample that has not been contacted with the test agent and identifying an agent as a candidate agent that restores LRP cleavage and/or processing if the cleavage and/or processing of LRP and/or fragment(s) thereof differs in the test and control samples; or is substantially similar; wherein the positive control sample contains LRP and/or fragment(s) thereof and a presenilin and/or fragment(s) thereof that is not associated with an altered processing of LRP. The presenilin and/or fragment(s) thereof in the positive control sample can be a wild-type presenilin. The cleavage or processing of LRP and/or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that has a molecular weight of about 20 kD. The LRP fragment that has a molecular weight of about 20 kD can contain an amino acid sequence that is contained within a transmembrane region of LRP; or can bind with an antibody generated against a C-terminal amino acid sequence of an LRP. The C-terminal amino acid sequence of LRP is a sequence of about the C-terminal 13 amino acids of an LRP. The LRP fragment that has a molecular weight of about 20 kD can comprise an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10; can be one that is present when an LRP is not cleaved by a presenilin-dependent activity; or can occur in the presence of an inhibitor of a presenilin-dependent activity. In one embodiment, the inhibitor is DAPT. The LRP processing can be assessed by determining the presence or absence and/or level of an LRP C-terminal fragment (CTF); or by determining the presence or absence and/or level of a fragment of LRP that binds to an antibody. The antibody can bind to an epitope in the C-terminal about 13 amino acids of an LRP, and can be a polyclonal antibody. The sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample. In one embodiment, the sample can comprise a cell that contains the presenilin, LRP

and/or fragment(s) of presenilin and/or LRP. The cell can be eukaryotic, mammalian, rodent or a human cell.

Also provided herein are methods for modulating LRP, comprising altering the structure, function and/or activity of a presenilin, and/or fragment(s) thereof, in a sample comprising LRP, and/or fragment(s) thereof, and a presenilin, and/or fragment(s) thereof, whereby the LRP is modulated. In another embodiment, provided herein are methods for modulating LRP, comprising contacting a sample comprising an LRP, and/or fragment(s) thereof, and presenilin, and/or fragment(s) thereof, with an agent that modulates the presenilin and/or fragment(s) thereof or a presenilin-dependent activity, whereby LRP is modulated. In these methods the cleavage, processing, structure, function and/or activity of LRP can be modulated. The method can further comprise selecting a sample for modulation of LRP. The sample can comprise a composition selected from the group consisting of a cell, tissue, organism, cell or tissue lysate, cell or tissue extract, a cell membrane, a membrane preparation from a cell and a cell-free sample.

Also provide herein are methods for identifying an agent that modulates Aβ42 levels, comprising comparing the levels of bound antibody and/or fragment(s) thereof in a test sample contacted with the test agent and a control sample not contacted with the test agent; and identifying an agent as an agent that modulates Aβ42 levels if the levels of bound antibody differ in the test and control samples; wherein the sample comprises APP or portion(s) thereof; and the antibody and/or fragment(s) thereof comprises a sequence of amino acids selected from the group consisting of amino acids 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94 and 1-95 of SEQ ID NO: 12 and any amino acid sequences containing modifications of these amino acid sequences that retain the antigen-binding properties of an antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14. In another embodiment, provided herein are methods for identifying an agent that modulates Aβ42 levels, comprising comparing the levels of bound antibody and/or fragment(s) thereof in a test sample contacted with the test agent and a control sample not contacted with the test agent; and identifying an agent as an agent that modulates Aβ42 levels if the levels of bound antibody differ in the test and control samples;

wherein the sample comprises APP or portion(s) thereof; and the antibody and/or fragment(s) thereof comprises a sequence of amino acids selected from the group consisting of amino acids 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96 and 1-97 of SEQ ID NO: 14 and any amino acid sequences containing modifications of these amino acid sequences that retain the antigen-binding properties of an antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14. In these methods, the antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14 can be an IgG. The antibody and/or fragment(s) thereof can bind Aβ42 without substantially binding other Aβ forms, such as Aβ40. The antibody and/or fragment(s) thereof can have at least about 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity for Aβ42 relative to other forms of Aβ, such as Aβ40. In addition, the antibody and/or fragment(s) thereof can have an affinity constant for binding to Aβ42 of at least about 10^5 l/mol, 2 x 10^5 l/mol, 3 x 10^5 l/mol, 4 x 10^5 l/mol, 5 x 10^5 l/mol, 6 x 10^5 l/mol, 7 x 10^5 l/mol, 8 x 10^5 l/mol, 9 x 10^5 l/mol, 10^6 l/mol, 2 x 10^6 l/mol, 3 x 10^6 l/mol or 4 x 10^6 l/mol or more. In one embodiment, the agent identified as an agent that modulates Aβ42 levels can reduce Aβ42 levels. The concentration of test agent can be less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. The step of identifying an agent as an agent that modulates Aβ42 levels can comprise identifying an agent that reduces Aβ42 levels with and IC_{50} of about 25 μM or less or about 20 μM or less.

Also provided herein are methods for identifying an agent that modulates Aβ levels, comprising assessing a test agent that modulates Aβ42 levels to determine if it modulates the level of one or more other Aβ peptides; and identifying an agent that modulates Aβ42 levels to a greater extent than it modulates the level of one or more other Aβ peptides. The step of identifying can comprise identifying an agent that modulates Aβ42 levels without substantially altering the level of one or more other Aβ peptides, such as Aβ40. The step of identifying can comprise identifying an agent that modulates Aβ42 levels to a greater extent than it modulates the level of Aβ40. In one

embodiment, the test agent reduces Aβ42 levels. In another embodiment, the test agent increases Aβ42 levels.

Further provided herein are methods for identifying an agent that modulates Aβ levels, comprising assessing a test agent that modulates Aβ42 levels to determine if it modulates the level of one or more other Aβ peptides; and identifying an agent that modulates Aβ42 levels and Aβ39 levels. The test agent can reduce Aβ42 levels or can increase Aβ42 levels. The step of identifying can comprise identifying an agent that increases Aβ39 or that reduces Aβ39. In another embodiment, the step of identifying can comprise identifying an agent that modulates Aβ42 levels and Aβ39 levels to a greater extent than it modulates Aβ40 levels. The step of identifying can comprise identifying an agent that modulates Aβ42 levels and Aβ39 without substantially altering the level of Aβ40. In one embodiment, the step of assessing a test agent can comprise comparing the levels of one or more Aβ peptides other than Aβ42 in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent as an agent that modulates Aβ42 levels to a greater extent than it modulates the level of one or more other Aβ peptides if the difference in the levels of one or more Aβ peptides other than Aβ42 in the test and control samples is less than the difference in the Aβ42 levels of the test and control samples. In another embodiment, the step of assessing a test agent can comprise comparing the levels of one or more Aβ peptides other than Aβ42 in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent as an agent that modulates Aβ42 levels to a greater extent than it modulates the level of one or more other Aβ peptides if the levels of one or more Aβ peptides other than Aβ42 in the test and control samples are substantially unchanged. In another embodiment, the step of assessing a test agent can comprise comparing the levels of Aβ39 in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent as an agent that modulates Aβ42 levels and Aβ39 levels if Aβ39 levels in the test and control samples differ.

Likewise, the step of identifying can comprise identifying an agent as an agent that modulates Aβ42 levels and Aβ39 levels to a greater extent than it modulates the level of Aβ40 if the difference in the levels of Aβ40 in a test sample that has been contacted with the test agent and a control sample that has not been contacted with a test agent is less than the difference in the Aβ42 and Aβ39 levels of the test and control samples. The step of identifying can also comprise identifying an agent as an agent that modulates Aβ42 levels and Aβ39 levels to a greater extent than it modulates the level Aβ40 if the levels of Aβ40 in test sample that has been contacted with test agent and a control sample that has not been contacted with test agent are substantially unchanged. The methods can further comprise a step of identifying the test agent as an agent that modulates Aβ42 levels; wherein the step of identifying the test agent as an agent that modulates Aβ42 levels is performed prior to or simultaneously with the step of assessing the test agent; and if the test agent is identified as an agent that modulates Aβ42 levels simultaneously with the step of assessing the test agent, then the step of assessing includes determining if the test agent modulates the level of Aβ42. The step of identifying the test agent as an agent that modulates Aβ42 levels can comprise comparing the levels of Aβ42 in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and identifying a test agent as an agent that modulates Aβ42 levels if the levels of Aβ42 in the test and control samples differ. The levels of Aβ42 in the samples are assessed in a method comprising an immunoassay wherein an antibody and/or fragment(s) thereof that bind Aβ42 without substantially binding other Aβ forms is used. The antibody and/or fragment(s) thereof can be at least about 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity for Aβ42 relative to other forms of Aβ, such as Aβ40. The antibody and/or fragment(s) thereof bind Aβ42 without substantially binding Aβ40. In one embodiment, the test agent can reduce Aβ42 levels. In the step of identifying the test agent as an agent that modulates Aβ42 levels, the concentration of test agent can be less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. The step of identifying the test agent as an agent that modulates Aβ42 levels can comprise identifying an agent that reduces Aβ42 levels with and IC_{50} of

about 25 μM or less or about 20 μM or less. The sample can comprise APP and/or portion(s) thereof. In other embodiments, the sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample. The sample can comprise a cell. The Aβ can be a cellular and/or extracellular Aβ.

Also provided herein are methods for modulating Aβ levels of a sample, comprising altering the Aβ peptide-producing cleavage of APP, the processing of APP, the processing of Aβ and/or the levels of Aβ such that the level of Aβ42 is modulated to a greater extent than the level of one or more other Aβ peptides is modulated. Also provided are methods for modulating Aβ levels of a sample, comprising contacting a sample comprising APP and/or a portion(s) thereof with an agent that modulates the level of Aβ42 to a greater extent than the level of one or more other Aβ peptides. The level of Aβ42 can be modulated without substantially altering the level of one or more other Aβ peptides. The level of Aβ42 can be modulated to a greater extent than the level of Aβ40. The level of Aβ42 is modulated without substantially altering the level of Aβ40. The level of Aβ42 can be reduced or increased. The level of Aβ42 and the level of Aβ39 can be modulated to a greater extent than the level of one or more other Aβ peptides, such as Aβ40. The level of Aβ42 and the level of Aβ39 can be modulated without substantially altering the level of one or more other Aβ peptides, such as Aβ40. In particular embodiments, the level of Aβ42 is reduced; the level of Aβ39 is increased; or the level of Aβ42 is increased. The concentration of the agent can be less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. The sample can comprise APP and/or portion(s) thereof. The Aβ can be a cellular and/or extracellular Aβ.

Also provided herein are methods for identifying an agent that modulates Aβ levels, comprising assessing a test agent that alters the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides to determine if it effects one or more presenilin-dependent activities other than the presenilin-dependent processing of APP or portion(s) thereof; and identifying an agent that modulates Aβ levels without substantially altering one or

more presenilin-dependent activities other than the presenilin-dependent processing of APP.

In the methods provided herein, the test agent can modulate Aβ42 levels, such as to a greater extent than it modulates the levels of other Aβ peptides; without substantially altering the level of one or more other Aβ peptides; to a greater extent than it modulates the levels of Aβ40; or without substantially altering the level of Aβ40. In another embodiment, the test agent can modulate Aβ42 and Aβ39 levels, such as to a greater extent than it modulates the levels of other Aβ peptides; without substantially altering the level of one or more other Aβ peptides; to a greater extent than it modulates the levels of Aβ40; or without substantially altering the level of Aβ40. In these methods, the step of assessing a test agent can comprise comparing one or more presenilin-dependent activities other than the presenilin-dependent processing of APP and/or portion(s) thereof in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent that modulates Aβ levels without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP if the one or more presenilin-dependent activities other than the presenilin-dependent processing of APP is (are) substantially unchanged in the test and control samples. The presenilin-dependent activity other than presenilin-dependent processing of APP can be the cleavage and/or processing of a substrate, and/or portion(s) thereof, other than APP. The test agent can reduce or increase Aβ42 levels.

Also provided herein are methods for identifying an agent that modulates Aβ levels, comprising assessing a test agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides to determine if it effects the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof other than APP or other than the presenilin-dependent processing of APP or portion(s) thereof; and identifying an agent that modulates Aβ levels without substantially altering the cleavage and/or processing of the presenilin substrate and/or portion(s) thereof that is other than APP. The step of assessing the test agent can comprise comparing (a) the cleavage and/or

processing of a presenilin substrate, and/or portion(s) thereof, other than APP, and/or (b) the levels of a fragment(s) of the presenilin substrate and/or portion(s) thereof in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent if the cleavage and/or processing of the presenilin substrate and/or portion(s) thereof and/or the levels of a fragment(s) of the presenilin substrate and/or portion(s) thereof in the test and control samples do not substantially differ. The step of assessing can comprise comparing (a) the cleavage and/or processing of a presenilin substrate, and/or portion(s) thereof, other than APP and/or (b) the levels of a fragment(s) of the presenilin substrate and/or portion(s) thereof in a test sample that has been contacted with the test agent and a positive control sample; and the step of identifying comprises identifying an agent if the cleavage and/or processing of a presenilin substrate, and/or portion(s) thereof, and/or the levels of fragment(s) of the presenilin substrate and/or portion(s) thereof in the test and positive control samples substantially differ; wherein the positive control sample is one that has been contacted with an modulator of presenilin and/or presenilin-dependent activity. The modulator of presenilin and/or presenilin-dependent activity is an inhibitor of presenilin and/or presenilin-dependent activity. The inhibitor can be DAPT. The level of a substrate fragment in the test sample can be less than about 40%, 35%, 30% or 20% of the level of the fragment in the positive control sample. The concentration of test agent is less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. The sample comprises a presenilin substrate and/or portion(s) thereof; and/or presenilin and/or portion(s) thereof. The method can further comprise a step of identifying the test agent as an agent that modulates Aβ levels; wherein the step of identifying the test agent as an agent that modulates Aβ levels is performed prior to or simultaneously with the step of assessing the test agent; and if the test agent is identified as an agent that modulates Aβ levels simultaneously with the step of assessing the test agent, then the step of assessing includes determining if the test agent modulates Aβ levels. The step of identifying the test agent as an agent that modulates the cleavage of APP and/or portion(s) thereof that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ

peptides can comprise comparing the Aβ peptide-producing cleavage of APP, or portion(s) thereof, APP processing, Aβ processing and/or Aβ levels in a test sample containing APP and/or portion(s) thereof that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and identifying an agent as an agent that modulates Aβ levels if the Aβ peptide-producing cleavage of APP, or portion(s) thereof, APP processing, Aβ processing and/or Aβ levels in the test and control samples differ. The presenilin substrate and/or portion(s) thereof can be selected from the group consisting of LRP, Notch, E-cadherin, Erb-B4, and portions of LRP, Notch, E-cadherin and Erb-B4. The step of assessing the test agent can comprise comparing the levels of an intracellular carboxyl domain fragment of Notch, E-cadherin and/or Erb-B4 in test and control samples; and the step of identifying comprises identifying an agent if the cleavage and/or processing of Notch, E-cadherin and/or Erb-B4 (and/or portion(s) thereof) and/or the levels of an intracellular carboxyl domain fragment of Notch, E-cadherin and/or Erb-B4 in the test and control samples do not substantially differ. The sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample.

Also provided herein are methods for identifying an agent that modulates Aβ levels, comprising assessing a test agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides to determine if it effects the cleavage and/or processing of LRP and/or portion(s) thereof; and identifying an agent that modulates Aβ levels without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof. The test agent can modulate Aβ42 levels; modulate Aβ42 levels to a greater extent than it modulates the levels of other Aβ peptides; modulate Aβ42 levels without substantially altering the level of one or more other Aβ peptides; modulate Aβ42 levels to a greater extent than it modulates the levels of Aβ40; modulate Aβ42 levels without substantially altering the level of Aβ40; modulate Aβ42 and Aβ39 levels; modulate Aβ42 and Aβ39 levels to a greater extent than it modulates the levels of other

$A\beta$ peptides; modulate $A\beta42$ and $A\beta39$ levels without substantially altering the level of one or more other $A\beta$ peptides; modulate $A\beta42$ and $A\beta39$ levels to a greater extent than it modulates the levels of $A\beta40$; modulate $A\beta42$ and $A\beta39$ levels without substantially altering the level of $A\beta40$; reduces $A\beta42$ levels; increases $A\beta39$ levels. The step of assessing the test agent can comprise comparing (a) the cleavage and/or processing of LRP, and/or portion(s) thereof, and/or (b) the levels of a fragment(s) of LRP and/or portion(s) thereof in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent if the cleavage and/or processing of LRP and/or portion(s) thereof and/or the levels of a fragment(s) of LRP and/or portion(s) thereof in the test and control samples do not substantially differ. The step of assessing can comprise comparing (a) the cleavage and/or processing of LRP, and/or portion(s) thereof, and/or (b) the levels of a fragment(s) of LRP and/or portion(s) thereof in a test sample that has been contacted with the test agent and a positive control sample; and the step of identifying comprises identifying an agent if the cleavage and/or processing of LRP, and/or portion(s) thereof, and/or the levels of fragment(s) of LRP and/or portion(s) thereof in the test and positive control samples substantially differ; wherein the positive control sample is one that has been contacted with an modulator of presenilin and/or presenilin-dependent activity. In one embodiment, the modulator of presenilin and/or presenilin-dependent activity can be an inhibitor of presenilin and/or presenilin-dependent activity. The level of an LRP fragment in the test sample can be less than about 40%, 35%, 30% or 20% of the level of the fragment in the positive control sample. The concentration of test agent can be less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. The cleavage and/or processing of LRP, and/or portion(s) thereof, and/or the levels of a fragment(s) of LRP and/or portion(s) thereof can be assessed by determining the presence, absence and/or level of one or more fragments of LRP and/or the composition of LRP. The cleavage and/or processing of LRP, and/or portion(s) thereof, and/or the levels of a fragment(s) of LRP and/or portion(s) thereof can be assessed by determining the presence, absence and/or level of an LRP fragment that has a molecular weight of about 20 kD. The LRP fragment that has a molecular weight

of about 20 kD can contain an amino acid sequence that is contained within a transmembrane region of LRP; can bind with an antibody generated against a C-terminal amino acid sequence of an LRP, wherein the C-terminal amino acid sequence of LRP is a sequence of about the C-terminal 13 amino acids of an LRP; can comprise an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10; is one that is present when an LRP is not cleaved by a presenilin-dependent activity; is one that occurs in the presence of an inhibitor of a presenilin-dependent activity. The cleavage and/or processing of LRP, and/or portion(s) thereof, and/or the levels of a fragment(s) of LRP and/or portion(s) thereof can be assessed by determining the presence, absence and/or level of an LRP C-terminal fragment (CTF); can be assessed by determining the presence or absence and/or level of a fragment of LRP that binds to an antibody. The sample can comprise LRP and/or portion(s) thereof; or presenilin and/or portion(s) thereof. The method can further comprise a step of identifying the test agent as an agent that modulates Aβ levels; wherein the step of identifying the test agent as an agent that modulates Aβ levels is performed prior to or simultaneously with the step of assessing the test agent; and if the test agent is identified as an agent that modulates Aβ levels simultaneously with the step of assessing the test agent, then the step of assessing includes determining if the test agent modulates Aβ levels. The step of identifying the test agent as an agent that modulates the cleavage of APP and/or portion(s) thereof that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides can further comprise: comparing the Aβ peptide-producing cleavage of APP, or portion(s) thereof, APP processing, Aβ processing and/or Aβ levels in a test sample containing APP and/or portion(s) thereof that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and identifying an agent as an agent that modulates Aβ levels if the Aβ peptide-producing cleavage of APP, or portion(s) thereof, APP processing, Aβ processing and/or Aβ levels in the test and control samples differ. The sample comprises a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free

extract or other cell-free sample. The step of identifying the test agent as an agent that modulates Aβ levels can comprise identifying an agent that reduces Aβ42 levels in test samples contacted with the test agent by greater than or equal to about 50% compared to the levels of Aβ42 in a control sample that has not been contacted with the agent. The concentration of the identified agent can be less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. In one embodiment, the step of identifying an agent that modulates Aβ levels without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof can comprise identifying an agent if the level of an ~20 kD fragment of LRP in a test sample is less than about 20% of the level of the fragment in a positive control sample that has been contacted with an inhibitor of presenilin and/or presenilin-dependent activity. In one embodiment, the Aβ levels are extracellular levels and the LRP fragment levels are cellular levels.

Further provided herein are methods of modulating the Aβ levels of a sample, comprising modulating the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP. In one embodiment, the modulating can be effected by contacting the sample with an agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP. Also provided are methods of modulating the Aβ levels of a sample, comprising modulating the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP. In one embodiment, the modulating can be effected by contacting the sample with an agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP.

Further provided are methods of modulating the Aβ levels of a sample, comprising modulating the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof, wherein modulating can be effected by contacting the sample with an agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof. In the above methods, the levels of Aβ42 can be modulated: to a greater extent than the levels of other Aβ peptides; without substantially altering the level of one or more other Aβ peptides; to a greater extent than the levels of Aβ40; without substantially altering the level of Aβ40; and the like. In other embodiments, the levels of Aβ42 and Aβ39 can be modulated, such as: to a greater extent than the levels of other Aβ peptides; without substantially altering the level of one or more other Aβ peptides; to a greater extent than the levels of Aβ40; without substantially altering the level of Aβ40. In other embodiments, the level of Aβ42 can be reduced or increased. Likewise, the level of Aβ39 can be increased or reduced. The sample can comprise presenilin and/or portion(s) thereof; APP and/or portion(s) thereof; a presenilin substrate and/or portion(s) thereof; and the like. The sample can comprise one or more of LRP, Notch, E-cadherin, TrkB, APLP2, hIre1α, Erb-B4, portion(s) of LRP, portion(s) of Notch, portion(s) of E-cadherin, portion(s) of TrkB, portion(s) of APLP2, portion(s) of hIre1α, and portion(s) of Erb-B4. The sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample. In one embodiment, the sample comprises a cell, such as a eukaryotic, a mammalian, a rodent or a human cell. The Aβ can be a cellular and/or extracellular Aβ. In particular embodiments of these methods, the Aβ42 levels of the sample can be reduced by greater than or equal to about 50%. The presenilin substrate and/or portion(s) thereof can be selected from the group consisting of Notch, E-cadherin, Erb-B4, and portions of Notch, E-cadherin and Erb-B4. In one embodiment, the levels of an

intracellular carboxyl domain fragment of Notch, E-cadherin and/or Erb-B4 are substantially unchanged. In another, the level or absence of an ~20 kD fragment of LRP is substantially unchanged. In other embodiments, the LRP fragment that has a molecular weight of about 20 kD: can contain an amino acid sequence that is contained within a transmembrane region of LRP; or can bind with an antibody generated against a C-terminal amino acid sequence of an LRP, wherein the C-terminal amino acid sequence of LRP is a sequence of about the C-terminal 13 amino acids of an LRP. In other embodiments, the LRP fragment that has a molecular weight of about 20 kD: can comprise an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10; can be one that is present when an LRP is not cleaved by a presenilin-dependent activity; can be one that occurs in the presence of an inhibitor of a presenilin-dependent activity. The inhibitor can be DAPT. In these methods, the level or absence of an LRP-CTF can be substantially unchanged. The concentration of agent can be less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. In a particular embodiment, the agent reduces Aβ42 levels with an IC_{50} of about 25 μM or less or about 20 μM or less.

Also provided herein are methods for treating or preventing a disease or disorder, comprising administering to a subject an agent that modulates the Aβ peptide-producing cleavage of APP, the processing of APP, the processing of Aβ and/or the levels of Aβ such that the level of Aβ42 is modulated to a greater extent than the level of one or more other Aβ peptides is modulated. The level of Aβ42 can be modulated: without substantially altering the level of one or more other Aβ peptides; to a greater extent than the level of Aβ40; without substantially altering the level of Aβ40. In one embodiment, the level of Aβ42 is reduced. In other embodiments, the level of Aβ42 and the level of Aβ39 can be modulated to a greater extent than the level of one or more other Aβ peptides; to a greater extent than the level of Aβ40; without substantially altering the level of one or more other Aβ peptides; without substantially altering the level of Aβ40. The level of Aβ42 can be reduced or increased.

Also provided herein are methods for treating or preventing a disease or disorder, comprising administering to a subject an agent that modulates the Aβ peptide-producing

cleavage of APP, the processing of APP, the processing of Aβ and/or the levels of Aβ without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP. Also provided are methods for treating or preventing a disease or disorder, comprising administering to a subject an agent that modulates the Aβ peptide-producing cleavage of APP, the processing of APP, the processing of Aβ and/or the levels of Aβ without substantially altering the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP.

Also provided herein are methods for treating or preventing a disease or disorder, comprising administering to a subject an agent that modulates the Aβ peptide-producing cleavage of APP, the processing of APP, the processing of Aβ and/or the levels of Aβ without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof. The level of Aβ42 can be modulated: to a greater extent than the levels of other Aβ peptides; to a greater extent than the levels of other Aβ peptides; without substantially altering the level of one or more other Aβ peptides; to a greater extent than the level of Aβ40; without substantially altering the level of Aβ40. In one embodiment, the level of Aβ42 is reduced. In other embodiments, the level of Aβ42 and the level of Aβ39 can be modulated: to a greater extent than the level of one or more other Aβ peptides; to a greater extent than the level of Aβ40; without substantially altering the level of one or more other Aβ peptides; without substantially altering the level of Aβ40. The level of Aβ42 can be reduced or increased. The presenilin substrate and/or portion(s) thereof can be selected from the group consisting of Notch, E-cadherin, Erb-B4, and portions of Notch, E-cadherin and Erb-B4.

With respect to any of the methods provided herein for treating a disease or disorder, the disease or disorder can be one characterized by altered Aβ production, catabolism, processing and/or levels. The disease or disorder can be one associated with amyloidosis, can be a neurodegenerative disease, and in a particular embodiment, is Alzheimer's disease.

Also provided are systems for use in assessing presenilin activity, comprising a source of presenilin activity; a source of LRP protein; and a reagent for determining LRP

protein composition. The reagent for determining LRP protein composition: can bind to LRP protein or a fragment of an LRP protein; can be an antibody or portion of an antibody that binds to LRP; can bind to a C-terminal portion of LRP; can bind to an ~20 kD fragment of LRP. The LRP fragment that has a molecular weight of about 20 kD; can contain an amino acid sequence that is contained within a transmembrane region of LRP; can bind with an antibody generated against a C-terminal amino acid sequence of an LRP. The C-terminal amino acid sequence of LRP can be a sequence of about the C-terminal 13 amino acids of an LRP. In other embodiments, the LRP fragment that has a molecular weight of about 20 kD: can comprise an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10; can be one that is present when an LRP is not cleaved by a presenilin-dependent activity; can be one that occurs in the presence of an inhibitor of a presenilin-dependent activity. The inhibitor can be DAPT. The source of a presenilin activity can be selected from the group consisting of a cell comprising a presenilin, an extract of a cell comprising a presenilin and medium comprising a presenilin.

Also provided herein are antibodies or fragments thereof comprising the sequence of amino acids 1-95 as set forth in SEQ ID NO: 12 and/or the sequence of amino acids 1-97 as set forth in SEQ ID NO: 14. The antibody or fragment thereof can comprise a light chain variable region that comprises the sequence of amino acids 1-95 as set forth in SEQ ID NO: 12. In another embodiment, the antibody or fragment thereof can comprise a light chain variable region that comprises the sequence of amino acids selected from the group consisting of 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, and 1-94 of SEQ ID NO: 12. The antibody or fragment thereof can comprise a heavy chain variable region that comprises the sequence of amino acids 1-97 as set forth in SEQ ID NO: 14. The antibody or fragment thereof can comprise a heavy chain variable region that comprises the sequence of amino acids selected from the group consisting of 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, and 1-96 of SEQ ID NO: 14. The antibodies or fragments thereof can further comprise one or more joining regions. In one embodiment, at least one joining region comprises the sequence of amino acids 96-107 as set forth in SEQ ID NO: 12. The antibody or fragment thereof can further comprise one or more

constant regions, wherein at least one constant region is a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63 and 65. In another embodiment, the at least one constant region is a human constant region.

The human constant region can comprise the sequence of amino acids as set forth in SEQ ID NO: 81. The at least one joining region can comprise the sequence of amino acids 98-118 as set forth in SEQ ID NO: 14. In this embodiment, the antibody or fragment thereof can further comprise one or more constant regions. The at least one constant region can be a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 69 and 71. The at least one constant region can be a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 83, 85 and 87. The at least one joining region can comprise a mouse joining region. The mouse joining region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 46, 48, 50, 52, 54, 55, 57, 59, 61 and 67. The antibody or fragment thereof can further comprise one or more constant regions, wherein at least one constant region is a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63, 65, 69 and 71. The at least one constant region can be a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 81, 83, 85 and 87. In another embodiment, the at least one joining region can comprise a human joining region. The human joining region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 73, 75, 77, 79, 89 and 91. The antibody or fragment thereof can further comprise one or more constant regions, wherein at least one constant region is a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63, 65, 69 and 71. The at least one constant region can be a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 81, 83, 85 and 87.

Also provide herein is an antibody or fragment thereof encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 11 and/or the sequence of nucleic acids as set forth in SEQ ID NO: 13. The antibody or fragment thereof can comprise: a light chain variable region encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 11; or a heavy chain variable region encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 13.

Also provided herein is an antibody or fragment thereof comprising the sequence of amino acids as set forth in SEQ ID NO: 97 and/or the sequence of amino acids as set forth in SEQ ID NO: 98. In embodiment, the antibody reacts with Aβ42 with an affinity of at least about 4 x 10^6 l/mol. In another embodiment, the antibody reacts with Aβ42 with an affinity of at least about 10^8 l/mol, or 10^9 l/mol or 10^{10} l/mol. The antibody or fragment thereof can comprise at least a portion of the antigen-binding region of the antibody, wherein the portion binds to the same antigenic determinant as the antibody with an affinity of at least about 1%, 5%, 10%, 50%, 70%, 80% or 100% of the entire antibody. Further provided is an antibody or fragment thereof comprising the sequence of amino acids 1-100 as set forth in SEQ ID NO: 16 and/or the sequence of amino acids 1-98 as set forth in SEQ ID NO: 18. The antibody or fragment thereof can comprise a light chain variable region that comprises the sequence of amino acids 1-100 as set forth in SEQ ID NO: 16. The antibody or fragment thereof can comprise a light chain variable region that comprises the sequence of amino acids selected from the group consisting of 1-50, 1-60, 1-70, 1-80, 1-90, 1-95, 1-96, 1-97, 1-98, and 1-99 of SEQ ID NO: 16. The antibody or fragment thereof can comprise a heavy chain variable region that comprises the sequence of amino acids 1-98 as set forth in SEQ ID NO: 18. The antibody or fragment thereof can comprise a heavy chain variable region that comprises the sequence of amino acids selected from the group consisting of 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, and 1-94, 1-95, 1-96, and 1-97 of SEQ ID NO: 18. The antibody or fragment thereof can further comprise one or more joining regions, wherein at least one joining region comprises the sequence of amino acids 101-112 as set forth in SEQ ID NO: 16. The antibody or fragment thereof can further comprise one or more constant regions. At least one constant region can be a mouse constant region. The mouse

constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63 and 65. In another embodiment, at least one constant region is a human constant region. The human constant region can comprise the sequence of amino acids as set forth in SEQ ID NO: 81. In another embodiment, at least one joining region can comprise the sequence of amino acids 99-114 as set forth in SEQ ID NO: 14. The antibody or fragment thereof can further comprise one or more constant regions, wherein at least one constant region is a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 69 and 71. In another embodiment, at least one constant region can be a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 83, 85 and 87. In one embodiment, at least one joining region comprises a mouse joining region. The mouse joining region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 46, 48, 50, 52, 54, 55, 57, 59, 61 and 67. The antibody or fragment thereof can further comprise one or more constant regions. In another embodiment, at least one constant region can be a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63, 65, 69 and 71. In another embodiment, at least one constant region is a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 81, 83, 85 and 87. At least one joining region can comprise a human joining region. The human joining region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 73, 75, 77, 79, 89 and 91. The antibody or fragment thereof can further comprise one or more constant regions, wherein at least one constant region is a mouse constant region or a human constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63, 65, 69 and 71. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 81, 83, 85 and 87.

Also provided herein is an antibody or fragment thereof encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 15 and/or the sequence of nucleic acids as set

forth in SEQ ID NO: 17. The antibody or fragment thereof can comprise a light chain variable region encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 15 and/or a heavy chain variable region encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 17. Also provided is an antibody or fragment thereof comprising the sequence of amino acids as set forth in SEQ ID NO: 99 and/or the sequence of amino acids as set forth in SEQ ID NO: 100.

Also provided herein is a protein or fragment thereof comprising the sequence of amino acids 1-95 as set forth in SEQ ID NO: 12 and/or the sequence of amino acids 1-97 as set forth in SEQ ID NO: 14. Further provided herein is a protein or fragment thereof comprising the sequence of amino acids as set forth in SEQ ID NO: 97 and/or the sequence of amino acids as set forth in SEQ ID NO: 98. Also provided is a protein or fragment thereof comprising the sequence of amino acids 1-100 as set forth in SEQ ID NO: 16 and/or the sequence of amino acids 1-98 as set forth in SEQ ID NO: 18. Further provided is a protein or fragment thereof comprising the sequence of amino acids as set forth in SEQ ID NO: 99 and/or the sequence of amino acids as set forth in SEQ ID NO: 100. Also provided herein is an isolated nucleic acid molecule that encoding these proteins. Also provided are isolated nucleic acid molecules that encode the antibodies provided herein.

Also provided herein are assays for determining the Aβ42 content of a sample, comprising contacting an antibody or fragment thereof provided herein with the sample under conditions whereby the antibody forms complexes with Aβ; and determining if the antibody or fragment thereof binds to a molecule in the sample. The Aβ can be Aβ42. The assay can be an enzyme-linked immunosorbant assay (ELISA). The antibody can be a capture antibody. The binding of the antibody or fragment thereof to a molecule in the sample can be determined by contacting the complex with a second antibody or fragment thereof, such as, for example an antibody or fragment thereof provided herein that contains the sequence of amino acids 1-100 of SEQ ID NO: 16 and/or the sequence of amino acids 1-98 of SEQ ID NO: 18.

Also provided herein is a kit containing a reagent for assessing cleavage of APP that produces one or more Aβ peptides, APP processing, Aβ processing and/or Aβ levels

and a reagent for assessing cleavage and/or processing of a presenilin substrate. In one embodiment, the presenilin substrate is LRP and/or portion(s) thereof. The reagent for assessing Aβ levels can be, for example, an antibody and/or fragment(s) thereof that specifically react with Aβ42, such as any of the Aβ42 specific antibodies provided herein. A reagent for assessing Aβ levels can include an antibody and/or fragment(s) thereof that reacts with two or more or most Aβ peptides, such as antibodies provided herein that contains the sequence of amino acids 1-100 of SEQ ID NO: 16 and/or the sequence of amino acids 1-98 of SEQ ID NO: 18. The reagent for assessing cleavage and/or processing of LRP can be an antibody and/or fragment(s) thereof that recognizes a fragment of LRP. The antibody can be one that prepared against the carboxyl-terminal 13 amino acid peptide of LRP (C-GRGPEDEIGDPLA). The LRP fragment can be one that is generated by a presenilin-dependent activity or a fragment that occurs in the absence of such activity. The fragment can have a molecular weight of about 20 kD.

Also provided is a method for identifying a candidate agent for the treatment or prophylaxis of a disease that includes steps of (a) contacting a sample that contains an altered test protein, and/or portion(s) thereof, and APP, and/or portion(s) thereof, with a test agent, wherein the altered protein is associated with altered Aβ42 production, catabolism, processing and/or Aβ42 levels; and (b) identifying a candidate agent that restores Aβ production, catabolism, processing and/or Aβ levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered Aβ42 production, catabolism, processing and/or Aβ42 levels without substantially altering the level of one or more other Aβ peptides. The method can be one wherein the candidate agent restores Aβ production, catabolism, processing and/or Aβ levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered Aβ42 production, catabolism, processing and/or Aβ42 levels without substantially altering the level of Aβ40. The method can be one wherein the candidate agent reduces the level of Aβ42 and/or increases Aβ39 levels. In one embodiment, the step of identifying a candidate agent comprises comparing Aβ production, catabolism, processing and/or Aβ levels in a test sample that has been contacted with test agent and a control sample that has not been

contacted with test agent and identifying an agent if Aβ production, catabolism, processing and/or Aβ levels in the test sample is such that Aβ42 levels differ in the test and control samples and the level of one or more other Aβ peptides is substantially unchanged in the test and control samples. The level of Aβ40 can be substantially unchanged in the test and control samples. The level of Aβ42 can be reduced in the test sample relative to the control sample. The level of Aβ39 can be increased. In another embodiment, the step of identifying comprises comparing Aβ production, catabolism, processing and/or Aβ levels in a test sample that has been contacted with the test agent and a positive control sample and identifying an agent as a candidate agent Aβ production, catabolism, processing and/or Aβ levels if Aβ production, catabolism, processing and/or Aβ levels in the test and control samples is substantially similar; wherein the positive control sample contains test protein and/or portion(s) thereof that is not associated with altered Aβ42 production, catabolism, processing and/or Aβ42 levels.

Another method provided herein for identifying a candidate agent for the treatment or prophylaxis of a disease includes steps of contacting a sample that contains an altered test protein, and/or portion(s) thereof, and APP, and/or portion(s) thereof, with a test agent, wherein the altered protein is associated with altered Aβ production, catabolism, processing and/or Aβ levels; and identifying a candidate agent that restores Aβ production, catabolism, processing and/or Aβ levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered Aβ production, catabolism, processing and/or Aβ levels without substantially altering (a) one or more presenilin-dependent activities other than the presenilin-dependent processing of APP, (b) the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP and/or (c) the cleavage and/or processing of LRP and/or portion(s) thereof. For example, the candidate agent can restore Aβ production, catabolism, processing and/or Aβ levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered Aβ production, catabolism, processing and/or Aβ levels without substantially altering the cleavage and/or processing of Notch, E-cadherin, Erb-B4 and/or portion(s) thereof. The candidate agent can reduce the level of Aβ42 and/or increase

Aβ39 levels. In one embodiment, the step of identifying a candidate agent comprises comparing Aβ production, catabolism, processing and/or Aβ levels in a test sample that has been contacted with test agent and a control sample that has not been contacted with test agent and identifying a candidate agent if Aβ production, catabolism, processing and/or Aβ levels in the test sample is such that Aβ42 levels differ in the test and control samples and one or more of the following is substantially similar in the test and control samples: (a) one or more presenilin-dependent activities other than the presenilin-dependent processing of APP, (b) the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP and/or (c) the cleavage and/or processing of LRP and/or portion(s) thereof. In a particular embodiment, the step of identifying comprises identifying a candidate agent that restores Aβ production, catabolism, processing and/or Aβ levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered Aβ production, catabolism, processing and/or Aβ levels without substantially altering the cleavage and/or processing of LRP. The altered protein in these methods can be one that is associated with altered Aβ42 production, catabolism, processing and/or Aβ42 levels; and the method can include identifying a candidate agent that restores Aβ production, catabolism, processing and/or Aβ42 levels.

In any of the methods provided herein for identifying a candidate agent for the treatment or prophylaxis of a disease, the altered test protein and/or portion(s) thereof can contain a mutation and/or can be altered relative to a wild-type protein, such as a wild-type protein encoded by a predominant allele or that occurs in an organism that exhibits normal Aβ42 production, catabolism, processing and/or Aβ42 levels. The mutation can be linked to familial Alzheimer's disease. In particular embodiments, the test protein is an APP or a presenilin. If the test protein is an APP, the APP, and/or portion(s) thereof, that is not an altered test protein does not have to be included in the sample. An altered APP or presenilin can be one that is linked to Alzheimer's disease.

The disease can be, for example, an amyloidosis-associated disease, a neurodegenerative disease, and, in particular, Alzheimer's Disease. For any of the methods, the sample can, for example, comprise a cell or organism. The cell can be, for

example, a eukaryotic cell, including a mammalian cell, such as, for example, a rodent or human cell. An organism may be, for example, a non-human transgenic animal.

Also provided are polypeptides comprising a sequence of amino acids that is selectively reactive with Aβ 42 and preferentially binds to low molecular weight forms of Aβ42. The polypeptide can comprise at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387. In one embodiment, the polypeptide comprises CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody A387. In another embodiment, the polypeptide comprises at least one CDR selected from the group consisting of amino acids 24-34 of SEQ ID NO:12, amino acids 50-56 of SEQ ID NO:12, amino acids 89-97 of SEQ ID NO:12, amino acids 26-35 of SEQ ID NO:14, amino acids 31-35 of SEQ ID NO:14, amino acids 26-31 of SEQ ID NO:14, amino acids 50-65 of SEQ ID NO:14, amino acids 50-58 of SEQ ID NO:14, and amino acids 98-107 of SEQ ID NO:14. The polypeptide can comprise at least a portion of a variable domain of the light chain or the heavy chain of an Aβ antibody. In one embodiment, the variable domain is selected from the group consisting of the light chain variable domain of A387, the heavy chain variable domain of A387, a polypeptide with at least 85% identity to the light chain variable domain of A387; a polypeptide with at least 85% identity to the heavy chain variable domain of A387.

The polypeptide can further comprise a scaffold. In one embodiment, the scaffold is a polypeptide scaffold. In one embodiment, the scaffold is a human polypeptide scaffold. In one embodiment, the scaffold is an antibody scaffold. The antibody scaffold can be selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment. The polypeptide can further comprise a detectable moiety. The polypeptide can further comprise a clearance domain. The clearance domain can be a ligand for an Fc receptor.

Also provided is a polypeptide, comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387. In one embodiment, the polypeptide comprises CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of

antibody A387. In one embodiment, the polypeptide comprises at least one CDR selected from the group consisting of amino acids 24-34 of SEQ ID NO:12, amino acids 50-56 of SEQ ID NO:12, amino acids 89-97 of SEQ ID NO:12, amino acids 26-35 of SEQ ID NO:14, amino acids 31-35 of SEQ ID NO:14, amino acids 26-31 of SEQ ID NO:14, amino acids 50-65 of SEQ ID NO:14, amino acids 50-58 of SEQ ID NO:14, and amino acids 98-107 of SEQ ID NO:14. The polypeptide can also be a chimeric polypeptide. The polypeptide can be an antibody.

The polypeptide can further comprising a clearance domain. The clearance domain can be a ligand for an Fc receptor. The polypeptide can further comprise a detectable moiety. The polypeptide can further comprising a scaffold. In one embodiment, the scaffold comprises a solid support. In another embodiment, the scaffold is a polypeptide scaffold. The scaffold can be a human polypeptide scaffold. The scaffold can be an antibody scaffold. The antibody scaffold can be selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment.

The polypeptide can comprise an amino acids 1-95 of SEQ ID NO:12, or a fragment thereof and/or comprises amino acids 1-97 of SEQ ID NO:14, or a fragment thereof. Such polypeptides can further comprise one or more joining regions. In one embodiment, the joining region comprises amino acids 96-107 of SEQ ID NO:12 or amino acids 98-118 of SEQ ID NO:14. In one embodiment, the joining region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 48, 50, 52, 54, 55, 57, 59, 61, 67, 73, 75, 77, 79, 89 and 91. The polypeptide can further comprising one or more constant regions. The constant region can be a mouse constant region. In one embodiment, the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:63, 65, 69 and 71. The constant region can also be a human constant region. In one embodiment, the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 83, 85 and 87. The polypeptide can comprise the amino acid sequence of SEQ ID NO:97 and/or SEQ ID NO:98.

The polypeptide can be specifically reactive with at least one Aβ. In one

embodiment, $A\beta$ is $A\beta42$. In one embodiment, the polypeptide binds $A\beta42$ without substantially binding other Aβpeptides.

Also provided is a polypeptide comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436. In one embodiment, the polypeptide comprisesCDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody B436. In one embodiment, the polypeptide comprises at least one CDR selected from the group consisting of amino acids 24-39 of SEQ ID NO:16, amino acids 55-61 of SEQ ID NO:16, amino acids 94-102 of SEQ ID NO:16, amino acids 26-35 of SEQ ID NO:18, amino acids 31-35 of SEQ ID NO:18, amino acids 26-31 of SEQ ID NO:18, amino acids 50-66 of SEQ ID NO:18, amino acids 50-59 of SEQ ID NO:18, and amino acids 99-103 of SEQ ID NO:18. The polypeptide can a chimeric polypeptide. The polypeptide can be an antibody.

The polypeptide can further comprise a scaffold. In one embodiment, the scaffold comprises a solid support. In one embodiment, the scaffold is a polypeptide scaffold. The scaffold can be a human polypeptide scaffold. The scaffold can be an antibody scaffold. The antibody scaffold can be selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment. The polypeptide can be specifically reactive with at least one $A\beta$ peptide.

The polypeptide can further comprise a clearance domain. The clearance domain can be a ligand for an Fc receptor. The polypeptide can further comprise a detectable moiety.

The polypeptide can comprise amino acids 1-100 of SEQ ID NO:16, or a fragment thereof and/or comprises amino acids 1-98 of SEQ ID NO:18, or a fragment thereof. Such polypeptides can further comprise one or more joining regions. In one embodiment, the joining region can comprise amino acids 101-112 of SEQ ID NO:16 or amino acids 99-114 of SEQ ID NO:18. In one embodiment, the joining region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 48, 50, 52, 54, 55, 57, 59, 61, 67, 73, 75, 77, 79, 89 and 91. The polypeptide can further comprising one or more constant regions. In one embodiment, the constant region is a

mouse constant region. The mouse constant region can comprise an amino acid sequence selected from the group consisting of SEQ ID NOS:63, 65, 69 and 71. In one embodiment, the constant region is a human constant region. The human constant region can comprise an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 83, 85 and 87. The polypeptide can comprise the amino acid sequence of SEQ ID NO:99 and/or SEQ ID NO:100.

Also provided are nucleic acid molecules encoding polypeptides provided herein. In one embodiment, the nucleic acid molecule encodes a polypeptide comprising a sequence of amino acids that is selectively reactive with Aβ 42 and preferentially binds to low molecular weight forms of Aβ42. In one embodiment, the nucleic acid molecule encodes a polypeptide comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387. Also provided are nucleic acid molecules encoding a polypeptide comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436. Also provided are kits comprising the polypeptides described herein.

Further provided are methods for assessing the presence or amount of Aβ in a sample, comprising contacting a polypeptide provided herein with the sample under conditions whereby a complex is formed between the polypeptide and Aβ, and assessing the presence or amount of the complex in the sample, and thereby determining the presence or amount of Aβ in the sample. The sample can be selected from the group consisting of a cell extract, extracellular medium, plasma, cerebrospinal fluid and brain. The presence or amount of the complex can be assessed using an enzyme-linked immunosorbent assay (ELISA).

Also provided are methods comprising administering to a subject a polypeptide provided herein. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387. In one embodiment, the polypeptide is selectively reactive with Aβ42 and preferentially binds to

low molecular weight forms of Aβ42. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436. In one embodiment, the subject has, or is at risk of developing, a disease associated with accumulation of Aβ. The disease can be Alzheimer's disease.

Also provided are methods of binding Aβ comprising administering to a subject a polypeptide provided herein to bind Aβ. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387 wherein the polypeptide is specifically reactive with at least one Aβ peptide. In one embodiment, the polypeptide is selectively reactive with Aβ42 and preferentially binds to low molecular weight forms of Aβ42. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436, wherein the polypeptide is specifically reactive with at least one Aβ peptide. In one embodiment, the subject has, or is at risk of developing, a disease associated with accumulation of Aβ. The disease can be Alzheimer's disease.

Also provided are methods of reducing Aβ level in an subject, comprising administering to the subject an effective amount of a polypeptide provided herein to reduce the level of at least one Aβ peptide. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387 wherein the polypeptide is specifically reactive with at least one Aβ peptide. In one embodiment, the polypeptide is selectively reactive with Aβ42 and preferentially binds to low molecular weight forms of Aβ42. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436, wherein the polypeptide is specifically reactive with at least one Aβ peptide. In one embodiment, the subject has, or is at risk of developing, a disease associated with accumulation of Aβ. The disease can be Alzheimer's disease. In one

embodiment, the level of at least one Aβ peptide in blood or plasma is reduced. In one embodiment, the level at least one Aβpeptide in brain is reduced.

Also provided are methods for identifying an agent that modulates Aβ levels, comprising comparing the levels of bound Aβ binding protein in a test sample contacted with the test agent and a control sample not contacted with the test agent; and identifying an agent as an agent that modulates Aβ levels if the levels of bound Aβ binding protein differ in the test and control samples; wherein the sample comprises APP or portion(s) thereof. The Aβ binding protein comprises a polypeptide provided herein. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387 wherein the polypeptide is specifically reactive with at least one Aβ peptide. In one embodiment, the polypeptide is selectively reactive with Aβ42 and preferentially binds to low molecular weight forms of Aβ42. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436, wherein the polypeptide is specifically reactive with at least one Aβ peptide.

Also provided are methods for identifying an agent that modulates Aβ42 levels, comprising, comparing the levels of bound Aβ binding protein in a test sample contacted with the test agent and a control sample not contacted with the test agent; and identifying an agent as an agent that modulates Aβ42 levels if the levels of bound Aβ binding protein differ in the test and control samples; whereinthe sample comprises APP or portion(s) thereof; and the Aβ binding protein comprises a sequence of amino acids selected from the group consisting of amino acids 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96 and 1-97 of SEQ ID NO: 12 and 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96 and 1-97 of SEQ ID NO: 14 and any amino acid sequences containing modifications of these amino acid sequences that retain the Aβ binding properties of an antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14.

Further provided herein are methods in the treatment or prophylaxis of disease

involving or characterized by Aβ and/or specific Aβ forms. In one embodiment, the method includes a step of administering a polypeptide provided herein to a subject having such a disease or disorder or predisposed to such a disease or disorder. In one embodiment, the disease is Alzheimer's disease. In one embodiment, Aβ42 levels are modulated. In one embodiment, the polypeptide is an Aβ binding protein or Aβ antibody. In one embodiment, the polypeptide comprising a sequence of amino acids that is selectively reactive with Aβ 42 and preferentially binds to low molecular weight forms of Aβ42.

DETAILED DESCRIPTION

A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications and sequences from GenBank and other data bases referred to herein are incorporated by reference in their entirety. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

As used herein, "Alzheimer's disease" or "AD" refers to a group of visible, detectable or otherwise measurable properties characteristic of AD. Exemplary properties include, but are not limited to, dementia, aphasia (language problems), apraxia (complex movement problems), agnosia (problems in identifying objects), progressive memory impairment, disordered cognitive function, altered behavior, including paranoia, delusions and loss of social appropriateness, progressive decline in language function, slowing of motor functions such as gait and coordination in later stages of AD, amyloid-containing plaques which are foci of extracellular amyloid-β (Aβ) protein deposition with dystrophic neurites and associated axonal and dendritic injury and microglia expressing surface antigens associated with activation (*e.g.*, CD45 and HLA-DR), diffuse ("preamyloid") plaques and neuronal cytoplasmic inclusions such as neurofibrillary

tangles containing hyperphosphorylated tau protein or Lewy bodies (containing α-synuclein). Standardized clinical criteria for the diagnosis of AD have been established by NINCDS/ADRDA (National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association) (McKhann *et al.* (1984) *Neurology 34*:939-944). The clinical manifestations of AD as set forth in these criteria are included within the definition of AD. For example, dementia may be established by clinical exam and documented by any of several neuropsychological tests, including the Mini Mental State Exam (MMSE) (Folstein and McHugh (1975) *J. Psychiatr. Res. 12*:196-198; Cockrell and Folstein (1988) *Psychopharm. Bull. 24*:689-692), the Blessed Test (Blessed *et al.* (1968) *Br. J. Psychiatry 114*:797-811), and the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-COG) Test (Rosen *et al.* (1984) *Am. J. Psychiatry 141*:1356-1364; Weyer *et al.* (1997) *Int. Psychogeriatr. 9*:123-138; and Ihl *et al.* (2000) *Neuropsychobiol. 4*:102-107).

As used herein, "amyloidosis" refers to a condition characterized by the presence of amyloid. Amyloid refers to a group of diverse but specific protein deposits observed in a number of different diseases. An example of an amyloid deposit is the β-amyloid plaque that is a defining pathological hallmark of Alzheimer's disease. The major protein component of the β-amyloid plaque is the Aβ peptide which is derived from processing of amyloid precursor protein (APP). Amyloid deposits, though diverse in their occurrence, can share some common morphologic properties. Many stain with specific dyes (*e.g.*, Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. Some share ultrastructural features and common x-ray diffraction and infrared spectra. Amyloidosis can be classified clinically as primary, secondary, familial and/or isolated. Primary amyloid appears de novo without any preceding disorder. Secondary amyloid is that form which appears as a complication of a previously existing disorder. Familial amyloid is a genetically inherited form found in particular geographic populations. Isolated forms of amyloid are those that tend to involve a single organ system.

An amyloidosis-associated disease is a disease involving accumulation of amyloid. Such diseases include, but are not limited to, AD, Down's syndrome, familial

amyloid polyneuropathy, familial amyloid cardiomyopathy (Danish type), isolated cardiac amyloid, amyloid angiopathy, systemic senile amyloidosis, idiopathic (primary) amyloidosis, reactive (secondary) amyloidosis, familial amyloidosis of Finnish type, and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) and Icelandic type.

As used herein, "amyloid precursor protein" or "APP" refers to a protein containing several characteristic domains, including a heparin-binding site, zinc- and copper-binding domains, a trophic domain containing an amino acid sequence (RERMS) that promotes fibroblast growth and a protease inhibitor domain for the matrix metalloprotease gelatinase A. Multiple isoforms of APP exist, typically distinguished by the number of amino acids in the particular isoform. Generally, most isoforms of APP are approximately 100 kD in molecular weight. Isoforms of APP include, for example, APP770 (which also contains a sequence homologous to the Kunitz family of serine protease inhibitors and a sequence homologous to the MRC OX-2 antigen), APP751 (the most abundant APP isoform in non-neuronal tissues), APP714, APP695 (the most abundant form in the brain), L-APP752, L-APP733, L-APP696, L-APP677, APP563 and APP365. All of the above-mentioned isoforms of APP, with the exception of APP563 and APP365, are transmembrane proteins that contain a single membrane-spanning domain and a long N-terminal extracellular (about two-thirds of the protein) and C-terminal cytoplasmic regions. APP563 and APP365 lack a transmembrane domain and are secreted. Examples of amino acid sequences for some of the APP isoforms are provided in SEQ ID NOs: 2 (APP770), 28 (APP751) and 30 (APP695). In addition, several mutations of the APP gene demonstrated in families with AD and other amyloidosis-associated diseases yield APP forms with varying amino acid sequences. Mutations of APP include those that result in a Val to Gly substitution at position 717 (V717G) of APP770 (the "London variant"), the "Swedish variant" double mutation at amino acid positions 670 and 671, with reference to the APP770 isoform, or positions 595 and 596, with reference to the APP695 isoform, in which a lysine is substituted with an asparagine and a methionine is substituted with a leucine, respectively, and a mutation at position 693 of the APP770 isoform that is associated with hereditary cerebral

hemorrhage with amyloidsis-Dutch type (HCHWA-D). Unless a specific isoform is specified, APP when used herein generally refers to any and all isoforms of APP.

As used herein, "cleavage" when used with reference to a substrate, *e.g.*, a protein, polypeptide, peptide, or fragment(s) thereof, refers to an alteration in the substrate structure. The alteration can be one resulting from, for example, an alteration, elimination or reduction of one or more interactions between elements within the substrate. In one example, if the substrate is a protein (polypeptide or peptide), cleavage of the substrate can be the degradation of the protein by a loss of one or more amino acids from the protein. The protein substrate, may, for example, be degraded into two or more fragments, each of which contains less than all the amino acids that the substrate contained. For instance, the processing of a larger precursor protein to yield a smaller mature protein can involve protein cleavage. Such cleavage can be, for example, the result of the hydrolysis of one or more peptide bonds in the protein. Thus, cleavage includes proteolytic cleavage of protein substrates. An alteration of a substrate structure due to cleavage (*e.g.*, the particular one or fragments generated upon cleavage of a protein substrate) can provide information relating to the types of compositions (*e.g.*, protease or proteolytic enzymes) and/or conditions or activities to which the substrate has been exposed.

As used herein, "processing" with reference to a protein, polypeptide or peptide refers to any post-translational modifications or alterations of the protein, polypeptide or peptide, such as may occur in maturation, degradation and/or clearance of such a molecule in a cell, and/or any post-translational packaging or transport of such a molecule through a pathway or process, such as a secretory pathway, uptake/internalization process, exo- or endocytosis, sequestration (*e.g.*, into a vesicle or endosome or lysosome) and clearance. In one example of processing, a protein, polypeptide or peptide can undergo cleavage, for example, to yield an active peptide from a larger inactive precursor protein, to liberate a functional fragment or peptide, such as a signaling peptide, or to degrade/digest a protein, polypeptide or peptide.

As used herein, "portion" and "fragment" are used interchangeably with reference to a protein, polypeptide or peptide and refer to a protein, polypeptide or peptide with a

primary structure that is less than or smaller than that of the protein, polypeptide or peptide of which it is a portion or fragment. For example, a fragment or portion of a protein can be a peptide generated upon cleavage of a larger precursor protein.

As used herein, "amyloid-β peptide" or "Aβ" refers to a peptide such as (a) a peptide that results from processing or cleavage of an APP and that is amyloidogenic, (b) one of the peptide constituents of β-amyloid plaques, (c) the 43-amino acid sequence set forth in SEQ ID NO: 4 or a fragment or portion thereof, and including substantially homologous sequences and/or (d) a fragment or portion of a peptide as set forth in (a) or (b). Aβ can also be referred to as βAP, AβP or βA4. Aβ peptides derived from proteolysis of APP generally are ~4.2 kD proteins and are typically 39 to 43 amino acids in length (see, *e.g.*, SEQ ID NO: 4 showing the 43-amino acid sequence of an Aβ peptide), depending on the carboxy-terminal end-point, which exhibits heterogeneity. However, Aβ peptides containing less than 39 amino acids, *e.g.*, Aβ39, Aβ38, Aβ37 and Aβ34, also can occur. Aβ peptides can be produced in an amyloidogenic APP processing pathway in which APP is cleaved by β-secretase (BACE) and one or more γ-secretase activities. Aβ peptides include those that begin at position 672 of APP770 (see SEQ ID NO: 2). Generally, as used herein, "Aβ peptide" includes any and all Aβ peptides, unless the amino acid residues are specified, such as, for example, 1-42 (Aβ42), 1-40 (Aβ40), 1-39 (Aβ39), 1-38 (Aβ38), 1-37 (Aβ37), 1-34 (Aβ34) and others.

As used herein "at least one Aβ peptide" refers to one or more species or sequence of amino acids of Aβ. For example, at least one Aβ peptide can be Aβ42, Aβ40, Aβ39, Aβ38, Aβ34, and combinations therof.

As used herein, "form of Aβ" or "Aβ form" refers to the conformational state of Aβ, for example monomers, oligomers such as dimers, trimers, pentamers, low molecular weight and high molecular weight oligomers of Aβ. Forms of Aβ also include aggregates, fibrils, tangles, and soluble Aβ. As used herein, "low molecular weight forms of Aβ" refers to monomers and low molecular weight oligomers of Aβ, including oligomers containing from about two to about 10 molecules of Aβ. As used herein, "high molecular weight forms of Aβ" refers to high molecular weight forms of Aβ such as aggregates of 50 or more Aβ molecules.

As used herein, "Aβ misregulation" refers to altered, abnormal or impaired Aβ regulation. For example, Aβ misregulation can be imbalances or disturbances in intracellular and/or secreted levels such as may result from altered Aβ production, clearance or degradation in a cell.

As used herein, "cellular" or "cell-associated" with reference to a molecule, such as, for example, a protein or peptide, refers to a molecule that is located within a cell (*e.g.*, in the cytoplasm or an intracellular organelle or vesicle) and/or at least partially associated with or in a cell membrane (*e.g.*, the plasma membrane or an intracellular membrane).

As used herein, "low-density lipoprotein receptor-related protein (LRP)" refers to a protein homologous to LRPs, which have been identified and described for a number of species, including several mammalian species. An example of an amino acid sequence of an LRP is provided in SEQ ID NO: 10. LRP proteins, which are discussed below in more detail, generally are cell surface receptors that bind and internalize a number of diverse extracellular ligands, including apolipoprotein E (apoE), α2-macroglobulin (α2M), APP, tissue-type plasminogen activator (tPA) and lactoferrin, for degradation by lysosomes. LRP expression is widespread; however, it is most highly expressed in the liver, brain and placenta. LRP is a member of the low-density lipoprotein receptor (LDLR) family. The extracellular region of receptors in this family contains several structural modules which include ligand-binding repeats of ~40 amino acids (including six cysteine residues forming three disulfide bonds), epidermal growth factor (EGF) precursor repeats (each also containing six cysteine residues), and modules with a consensus tetrapeptide (YWTD). In addition to these modules, these receptors contain a single transmembrane domain and a relatively short cytoplasmic tail with endocytosis signals and elements for interaction with cytoplasmic adaptor and scaffold proteins (*e.g.*, Dab, FE65, c-jun N-terminal kinase interacting proteins (JIPs) and postsynaptic density protein PSD-95) for mediating signal transduction.

As used herein, a "composition of low-density lipoprotein receptor-related protein (LRP)" refers to the make-up of LRP. The LRP may be LRP that is present anywhere, for example, in an analysis mixture, including an assay medium in which an analysis is

performed, an extracellular medium, or a cell membrane, lysate or extract. For example, a composition of LRP refers to the overall combination of any intact LRP protein(s), fragments thereof, sizes thereof, ratios and amounts thereof.

As used herein, "presenilin" refers to a protein homologous to the presenilin 1 (PS1) or presenilin 2 (PS2) proteins, and/or fragment(s) thereof, that have been identified and described for a number of species, including several mammalian species. Presenilins show a high degree of conservation between species, particularly of the hydrophobic structure. Examples of amino acid sequences of PS1 and PS2 proteins are provided in SEQ ID NOs: 6 and 8, respectively and in PCT Application Publication No. WO96/34099. Presenilin proteins generally are polytopic membrane proteins that can possess two or more aspartic acid residues within adjacent predicted transmembrane segments. Many presenilins possess protease-associated domains and are involved in a catalytic complex having catalytic activity. Presenilins can undergo proteolytic processing which can generate fragments, such as, for example, an ~35-kD N-terminal fragment and an ~20-25 kD C-terminal fragment. In vivo, the majority of detectable presenilin appears in the form of N- and C-terminal fragments that are tightly regulated and form a stable complex after processing. Thus, as used herein, "presenilin" refers to any full-length presenilin protein, presenilin proteins encoded by allelic and splice variants, and any fragments thereof, including biologically active fragments and functional units..

As used herein, "presenilin activity" or "presenilin-dependent activity" refers to an activity, such as a biological event or process, that is directly or indirectly influenced by a presenilin protein. An activity can be, for example, any biological, chemical, biochemical or molecular activity, including, but not limited to, interaction between molecules, such as binding between a protein or peptide and another molecule, a chemical reaction, *e.g.*, hydrolysis, and a cellular event, *e.g.*, secretion, endocytosis, signaling, molecular trafficking. A presenilin-dependent activity is influenced by a presenilin in such a way that the activity differs in the presence and absence of a presenilin. The difference can be, for example, a modification or alteration in the activity or a complete or near complete elimination of the activity. In a particular example, a

presenilin-dependent activity is an enzymatic activity. One such presenilin-dependent enzymatic activity is a presenilin-dependent proteolytic processing of APP, *e.g.*, γ-secretase cleavage of APP. Other presenilin-dependent enzymatic activities include, but are not limited to, cleavage of LRP, Notch, E-cadherin and Erb-B4.

As used herein, "presenilin substrate," "substrate for presenilin activity" and/or "substrate for presenilin-dependent enzyme activity" refers to a peptide, polypeptide, protein or fragment(s) thereof that is altered (*e.g.*, proteolytically processed, at least in part) in a presenilin-dependent manner. Thus, for example, in the case of a presenilin substrate that is altered by proteolytic processing of the substrate, if presenilin is absent, or presenilin activity is inhibited or reduced, the proteolytic processing of the presenilin substrate is altered, for example by an alteration in the levels and/or composition of fragments generated from the substrate, relative to the proteolytic processing of the substrate that occurs in the presence of normal (*e.g.*, wild-type) presenilin activity. Generally, a presenilin substrate can contain about one transmembrane domain, an ectodomain that is released or shed into the extracellular medium, and/or an intracellular domain. Exemplary presenilin substrates include, but are not limited to APP, LRP, Notch, TrkB, APLP2, hIre1α, E-cadherin and Erb-B4.

As used herein, "C-terminal fragment (CTF)" refers to a fragment of a protein that results from cleavage of the protein by a presenilin-dependent activity. For example, an LRP-CTF refers to a C-terminal fragment of LRP. When an LRP composition is assessed, for example, it can be evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage (or altered presenilin-dependent cleavage) of LRP is/are present and/or the level of any such fragment(s) produced. A presenilin-dependent cleavage described herein occurs within the C-terminal portion of LRP and within the β chain. Thus, a presenilin-dependent cleavage of LRP can be one, for example, that occurs in the C-terminal portion of LRP at a position C-terminal to amino acid position 3925 of SEQ ID NO: 10 (or of the amino acid sequence provided as GenBank Accession No. Q07954). The presenilin-dependent cleavage of LRP can be one that occurs within the sequence of the last approximately 580, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 25, or less amino acids of LRP. The presenilin-dependent cleavage can be one that

occurs C-terminal to the extracellular portion of the β chain (i.e., approximately amino acids 3944-4420 of SEQ ID NO: 10 or of the amino acid sequence provided as GenBank Accession No. Q07954); thus, C-terminal to amino acid 4420 of SEQ ID NO:10. The presenilin-dependent cleavage of LRP can be one that occurs near or within the region of the LRP protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of LRP can be one that generates a soluble intracellular peptide containing the extreme C-terminus of LRP and a membrane-associated peptide containing amino acid sequence of the transmembrane region of LRP, particularly the more C-terminal region of the transmembrane segment of LRP. Such fragments of LRP can be referred to as LRP-CTFs. Any LRP fragments generated by such presenilin-dependent activities have a molecular weight that is less than that of the β chain of LRP (β chain molecular weight is approximately 85-90 kD, or approximately 67 kD after deglycosylation with *N*-glycosidase F) and are encompassed by the term LRP-CTFs. Similarly, characteristic C-terminal fragments of APP are produced upon exposure to an a presenilin-dependent activity.

As used herein, "normal" with reference to a protein refers to a protein which performs its usual or normal physiological role and which is not causative of a disease or pathogenic condition. A normal gene or coding sequence is also one that is not causative of a disease or pathogenic condition and may encode a normal protein. The term normal is generally synonymous with wild-type. For any given gene, or corresponding protein, a number of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or disease.

As used herein, "mutant" with reference to a protein refers to a protein which does not perform it usual or normal physiological role, *e.g.*, it may be dysfunctional, and which can be associated with a disease or pathogenic state. A mutant gene generally is one that contains an alteration relative to a normal or wild-type gene such that it has altered function (*e.g.*, regulation or encoding of a mutant protein).

As used herein, "assess" and variations thereof refer to any type of evaluation, determination, observation, identification, detection, characterization and measurement,

whether quantitative, qualitative, comparative or relative.

As used herein, "determining the level of", "assessing the level of" and variations thereof with reference to a substance, such as, for example a peptide, protein or fragment thereof, can be determining the presence or absence of the substance and/or making a more quantitative assessment of level or amount of the substance.

As used herein, the term "polypeptide" is used interchangeably with the term "protein" and includes peptides of 2 or more amino acids. A polypeptide can be a single polypeptide chain, or to two or more polypeptide chains that are held together by non-covalent forces, by disulfide cross-links, or by other linkers (e.g. peptide linkers). Thus, a single heavy or light chain of an antibody, or an antibody fragment containing all or part of both heavy and light chains of an antibody, no matter how the chains are associated or joined, are exemplary molecules that are included within the term "a polypeptide." A polypeptide can contain non-proteinaceous components, such as sugars, lipids, detectable labels or therapeutic moieties. A polypeptide can be derivatized by chemical or enzymatic modifications (e.g. by replacement of hydrogen by an alkyl, acyl, or amino group; esterification of a carboxyl group with a suitable alkyl or aryl moiety; alkylation of a hydroxyl group to form an ether derivative; phosphorylation or dephosphorylation of a serine, threonine or tyrosine residue; or N- or O-linked glycosylation) or can contain substitutions of an L-configuration amino acid with a D-configuration counterpart.

As used herein, the term "chimeric polypeptide" refers to a polypeptide that contains amino acid residues from derived from two or more polypeptides or from one polypeptide but joined in different order from the original polypeptide. For example, a chimeric polypeptide can contain residues from related polypeptides from two or more species (e.g. CDR sequences from a mouse immunoglobulin (Ig), and a scaffold portion from a human Ig; or variable region residues from a mouse Ig, and constant region residues from a human Ig). A chimeric polypeptide also can contain residues from two or more unrelated polypeptides from the same or different species (e.g. CDR sequences from an Ig, and scaffold sequences from a lipocalin or Fn3 polypeptide).

As used herein, "antibody" refers to an immunoglobulin, whether natural or

partially or wholly synthetically produced, including any derivative thereof that retains the specific binding ability of the antibody. Hence antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin binding domain. Antibodies include members of any immunoglobulin chains, including IgG, IgM, IgA, IgD and IgE. As used herein, the term "antibody" includes, but is not limited to, polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, antibody fragments and antigen-binding fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included.

As used herein, "antibody fragment" refers to any derivative of an antibody that is less than full-length, retaining at least a portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', $F(ab)_2$, single-chain Fvs (scFv), FV, dsFv diabody and Fd fragments. The fragment can include multiple chains linked together, such as by disulfide bridges. An antibody fragment generally contains at least about 50 amino acids and typically at least 200 amino acids.

As used herein, an "Fv antibody fragment" is composed of one variable heavy domain (V_H) and one variable light domain linked by noncovalent interactions.

As used herein, a "dsFV" refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the V_H-V_L pair.

As used herein, an "$F(ab)_2$ fragment" is an antibody fragment that results from digestion of an immunoglobulin with pepsin at pH 4.0-4.5; it can be recombinantly expressed to produce the equivalent fragment.

As used herein, "Fab fragments" are antibody fragments that result from digestion of an immunoglobulin with papain; they can be recombinantly expressed to produce the equivalent fragment.

As used herein, "scFvs" refer to antibody fragments that contain a variable light chain (V_L) and variable heavy chain (V_H) covalently connected by a polypeptide linker in any order. The linker is of a length such that the two variable domains are bridged

without substantial interference. Included linkers are $(Gly-Ser)_n$ residues with some Glu or Lys residues dispersed throughout to increase solubility.

As used herein, "diabodies" are dimeric scFv; diabodies typically have shorter peptide linkers than scFvs, and they generally dimerize.

As used herein, the term "complementarity determining region" or "CDR" (also known as a "hypervariable region") refers to a region of an Ig molecule that varies greatly in amino acid sequence relative to flanking Ig sequences. The length and conformation of CDRs vary among Igs, but generally CDRs form short loops supported by a sandwich of two antiparallel beta-sheets. Three CDRs, designated CDR-L1, CDR-L2 and CDR-L3, are present in the variable region of an immunoglobulin light chain, and three CDRs, designated CDR-H1, CDR-H2 and CDR-H3, are present in the variable region of an immunoglobulin heavy chain. Each CDR generally contains at least one, and often several, amino acids residues that make contact with antigen, but all six CDRs are not necessarily required to maintain the binding specificity of an antibody.

As used herein, a "scaffold" refers to any structure that forms a conformationally stable structural support, or framework, which is able to display one or more sequences of amino acids (e.g. CDRs, a variable region, a binding domain) in a localized surface region. A scaffold can be a naturally occurring polypeptide or polypeptide "fold" (a structural motif), or can have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a naturally occurring polypeptide or fold. Exemplary modifications to a polypeptide that render it suitable for use as a scaffold include but are not limited to, deletions of those regions that form binding loops in the naturally-occurring molecule (e.g. deletions of the naturally-occurring CDRs); deletions of those regions that are unnecessary for structural integrity of the fold; substitutions of amino acids that flank the loop regions with residues that improve the properties of the polypeptide (such as improved affinity, specificity, or solubility; reduced immunogenicity, etc.); addition of detectable sequences, such as epitope tags. A scaffold can be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus. A scaffold can also be a solid support, such as a membranes, filters, chips, slides, wafers, fibers,

magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries, which is able to display one or more amino acid sequences (e.g. CDRs) in a localized surface region.

As used herein, the term "human polypeptide scaffold" refers to a polypeptide scaffold that is derived from a human polypeptide or has been engineered to resemble a human polypeptide. An example of a human polypeptide scaffold is a human antibody scaffold, which is used in a humanized antibody.

As used herein, the term "antibody scaffold" refers to a scaffold of an antibody that contains all or part of an immunoglobulin. Exemplary antibody scaffolds include whole antibodies, and fragments thereof, such as Fv fragments (which can or can not contain an introduced disulfide bond), Fab fragments, Fab' fragments, $F(ab')_2$ fragments, and single-chain scFv fragments. Antibody scaffolds also include all or part of an Ig heavy chain variable region, and all or part of an Ig light chain variable region.

As used herein, the term "clearance domain" refers to a domain that directly or indirectly mediates enhanced clearance of a polypeptide from the circulation. Thus, a polypeptide described herein as containing a "clearance domain" will have a shorter half-life in the circulation, alone and/or when bound to Aβ, than a polypeptide without such a domain.

As used herein, an "Aβ antibody" refers to an immunoglobulin, whether natural or partially or wholly synthetically produced, including any derivative thereof that is specifically reactive with at least one Aβ.

As used herein, an "Aβ binding protein" refers to a polypeptide, peptide or protein that is specifically reactive with at least one Aβ peptide. An Aβ binding protein can be an Aβ antibody or fragment(s) thereof. Aβ proteins also include chimeric polypeptides. For example, an Aβ binding protein can be a chimeric polypeptide that has the ability to bind Aβ displayed in a scaffold. An Aβ binding protein can also be derived de novo by screening for peptides, polypeptides and proteins that have the ability to bind at least one Aβ.

As used herein, "grafting" with respect to polypeptides refers to the construction of a chimeric polypeptide by covalently joining a peptide, protein or domain of a protein

to a scaffold.

As used herein, "operatively linked" (or, sequences that are in "operative association") indicates that the recited nucleotide sequences are positioned such that there is a functional relationship between the sequences in the context of transcription. For example, an Aβ binding protein nucleotide sequence, a promoter sequence and a reporter sequence can be in operative association if transcription of the reporter nucleic acid sequence can occur under control of the promoter sequence as modulated by the effect of the Aβ binding protein nucleotide sequence. When the Aβ binding protein nucleotide sequence comprises the promoter, the sequences can be in operative association if transcription of the reporter nucleic acid sequence can occur under control of the Aβ binding protein nucleotide sequence. Two sequences that are "operatively linked" are not necessarily contiguous.

As used herein, an "expression construct" refers to a nucleotide sequence with the capacity to express an mRNA or protein. Generally, expression constructs have a sequence of nucleotides encoding the mRNA and/or protein to be expressed, operatively linked to a promoter sequence.

As used herein a "detectable moiety" refers to a molecule that can be detected by visible, enzymatic, physical or chemical means. Detectable moieties include, but are not limited to, reporter genes or fragments thereof, enzymes or portions thereof and radiolabels. Exemplary detectable moieties include fluorescent proteins such as green, red and blue fluorescent proteins, β-galactosidase, alkaline phosphatase and radiolabels such as ^{125}I, ^{131}I, ^{213}Bi, ^{99}mTc, ^{111}In, ^{90}Y, and ^{32}P. Detectable moieties also include moieties that can be detected physical means such as detection of molecular weight by mass spectrometry and tags that can be detected such as a His_6 tag for metal binding or an epitope tag for antibody recognition.

As used herein, "humanized antibodies" refer to antibodies that are modified to include human sequences of amino acids so that administration to a human does not provoke an immune response, or provokes a milder immune response than a non-humanized antibody. Methods for preparation of such antibodies are known. For example, to produce such antibodies, the encoding nucleic acid in the hybridoma or other

prokaryotic or eukaryotic cell, such as an *E. coli* or a CHO cell, that expresses the monoclonal antibody is altered by recombinant nucleic acid techniques to express an antibody in which the amino acid composition is based on human antibodies.

As used herein, "specifically reactive," "specificity," "selectivity," "selective" and variations thereof in the context of an antibody binding an antigen refers to the degree of affinity an antibody has for a target antigen and the degree of discrimination between the target antigen and other, chemically similar structures. Antibodies and proteins, such as Aβ binding proteins, are determined to be specifically reactive if: 1) they exhibit a threshold level of binding affinity, and/or 2) they do not significantly cross-react with related polypeptide molecules. Antibodies and Aβ binding proteins herein are determined to be specifically reactive if they bind the target epitope with an affinity constant in the range of about 10^5 l/mole to 10^{12} l/mole, generally about 10^6 to 10^8 l/mole. In one embodiment, an antibody or Aβ binding protein is determined to be specifically reactive if it binds the target epitope with an affinity constant of at least about 10^5 l/mol, or at least about 10^6 l/mol. In a particular embodiment, an antibody or Aβ binding protein is determined to be specifically reactive if it binds the target epitope with an affinity constant of at least about 2 x 10^6 l/mol, or at least about 3 x 10^6 l/mol, or at least about 4 x 10^6 l/mol. The binding affinity of an antibody and an Aβ binding protein can be readily determined by one of skill in the art (Scatchard (1949) *Ann. N.Y. Acad. Sci. 51*: 660-672).

Selectivity of an antibody and an Aβ binding protein can refer to the degree of recognition of an antibody or Aβ binding protein for an antigen relative to other, particularly related, peptides or proteins. Selectivity or selectively reactive is considered a measure of the functional ability of an antibody to discriminate between the target antigen and other, chemically similar structures. In one aspect, selectivity of an antibody for a particular antigen relative to another peptide or protein can be determined by comparing the binding affinities of the antibody for the antigen and the other peptide. If the binding affinity (*e.g.*, as represented by an affinity constant) for the antigen is, for example, 1000-fold higher than that for the other peptide, the antibody can be said to be 1000-fold more selective or selectively reactive, for the antigen relative to the other

peptide.

As used herein, "bind preferentially" refers to the affinity of an Aβ binding protein, such as an Aβ antibody, for one antigen (such as an Aβ peptide or form) relative to another. For example, an Aβ binding protein can preferentially bind one Aβ form relative to another Aβ form, such as preferentially binding low molecular weight forms of Aβ relative to high molecular weight forms of Aβ. In one embodiment, an Aβ binding protein binds preferentially to a particular Aβ form relative to another Aβ form if the Aβ binding protein binds the particular Aβ form with at least 2-fold higher affinity as compared with binding to the other Aβ form. In another embodiment, an Aβ binding protein binds preferentially to a particular Aβ form relative to another Aβ form if the Aβ binding protein binds the particular Aβ form with at least 5-fold, 10-fold or more, including 20-fold and 100-fold higher affinity as compared with binding to the other Aβ form. In another embodiment, an Aβ binding protein binds preferentially to a particular Aβ peptide or form relative to another Aβ peptide or form if the binding of the Aβ binding protein to the particular Aβ peptide or form can be detected in an immuno assay, such as western blot or ELISA assay, but the binding of the Aβ binding protein to another Aβ peptide or form is substantially less in the same or a similar assay. As used herein, "modulation" with reference to Aβ levels refers to any alteration or adjustment in cellular and/or extracellular or secreted Aβ, including, but not limited to, alteration of Aβ concentration in the cytoplasm, cellular membranes, extracellular medium and/or intracellular organelles, *e.g.*, endoplasmic reticulum, endosome and lysosome, and any alteration of the production, clearance, and/or degradation of Aβ.

As used herein, "agent that modulates Aβ levels" refers to any substance that can modulate Aβ levels. Examples of agents include, but are not limited to, small organic molecules, amino acids, peptides, polypeptides, nucleotides, nucleic acids, polynucleotides, carbohydrates, lipids, lipoproteins, glycoproteins, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Agents may be obtained from a wide variety of sources including libraries of synthetic or natural compounds. Libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural

or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, acidification, etc. to produce structural analogs.

As used herein, "test agent", in the context of methods for identifying agents that modulate Aβ levels, refers to any substance that is being evaluated as a possible agent that modulates Aβ levels.

As used herein, "amelioration" refers to an improvement in a disease or condition or at least a partial relief of symptoms associated with a disease or condition.

As used herein, "substantially unchanged" or "without substantially altering or affecting" and variations thereof are used with reference to a particular composition, activity and/or process that is not a target for modulation. These expressions refer to the state (which includes amount or level) of the non-target composition, activity and/or process under specified differing conditions. A composition may be, for example, a particular protein or peptide, such as an Aβ peptide, or a fragment or peptide generated by cleavage of a protein, such as a presenilin substrate. An activity or process may be, for example, the cleavage or processing of a protein such as a presenilin substrate. Differing conditions include any physical, chemical, environmental or other conditions in which the composition, activity and/or process occurs. For example, differing conditions can be in the presence and absence of a test agent or agent that modulates a target composition, activity or process. A non-target composition, activity and/or process is substantially unchanged or is not substantially altered or affected if any variation in the composition, activity and/or process that occurs under specified differing conditions is an acceptable variation. Those of skill in the art can identify acceptable variation. For example, acceptable variation generally can be any alteration in the composition, activity and/or process (including, *e.g.*, increase or decrease in the amount or level) that is less than or relatively minimal in comparison to the variation in a target composition, proeess or activity under the specified differing conditions, or that is not associated with an undesired effect. An undesired effect can be, for example, an adverse effect on a

biological composition, cell, tissue, system or organism including or containing the cell or composition. Undesired effects include, for example, deleterious alterations in any aspect of cell function, decreased cell viability and cell death. Acceptable variation can also be any alteration in the composition, activity and/or process that is inconsequential (or without significant consequence) to an overall or ultimate downstream function in which the composition, activity and/or process is involved. Thus, in a particular example of a peptide that is not a target for modulation, substantially unchanged with respect to the levels of such a non-target peptide in the presence and absence of an agent being tested as a possible modulator of a target peptide means that there is no change, or an acceptable variation, in the level of the non-target peptide in the presence of the agent compared to in the absence of the agent. Acceptable variation in a non-target composition, activity and/or process (including levels or amounts) may be different for different compositions, activities and processes, and in the context of different sets of specified differing conditions. In some particular instances, acceptable variation can range from equal to or less than about 40, 30, 20, or 10% variation when compared under differing conditions, *e.g.*, in the presence and absence of a test agent. It should be understood that this definition of "substantially unchanged" or "without substantially altering or affecting" applies and is used with reference to a composition, activity and/or process that is not a target for modulation. In contrast, any variation (and particularly a statistically significant variation) in a composition, activity and/or process that is a target for modulation in the presence and absence of a test agent can be a sufficient modulation.

As used herein, "avidity" refers to the functional affinity or combining strength of an antibody with its antigen and is related to both the affinity of the reaction between the epitopes and paratopes, and the valences or recognition sites of the antibody and antigen.

As used herein, "selective modulation of Aβ levels" refers to the modulation of the levels of one or more forms of Aβ, wherein one or more other specified compositions or specified activities, processes or mechanisms are substantially unchanged, or without substantially altering or affecting one or more other specified compositions or specified activities, processes or mechanisms. For example, selective modulation of an Aβ peptide

can be relative to one or more other related polypeptide molecules (*e.g.*, other Aβ peptides) in which the level of a particular Aβ peptide is modulated without substantially altering the levels of one or more other Aβ peptides. In another example, selective modulation of an Aβ peptide can be relative to the processing of a presenilin substrate other than APP, in which Aβ levels are modulated without substantially altering the cleavage of the presenilin substrate that is other than APP.

As used herein, "related peptide molecules" refers to any peptide molecules with chemically similar structures, any peptides molecules that undergo similar processing by the same or similar enzymes, any peptide molecules derived from the same or similar precursor peptide molecule, and/or any peptide molecules that have the same or similar activities and/or functions.

As used herein, "treatment" means any manner in which the symptoms of a conditions, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein.

As used herein, a "combination" refers to any association between two or among more items.

As used herein, an "agent identified by the screening methods provided herein for identifying candidate agents for the treatment and/or prevention of a disease or disorder" refers to any compound that is a candidate for use as a therapeutic or as lead compound for design of a therapeutic. Such compounds can be small molecules, including small organic molecules, peptides, peptide mimetics, antisense molecules or dsRNA, such as RNAi, antibodies, fragments of antibodies, recombinant antibodies and other such compound which can serve as drug candidate or lead compound.

As used herein, a "peptidomimetic" is a compound that mimics the conformation and certain stereochemical features of the biologically active form of a particular peptide. In general, peptidomimetics are designed to mimic certain desirable properties of a compound, but not the undesirable properties, such as flexibility, that lead to a loss of a biologically active conformation and bond breakdown. Peptidomimetics may be prepared from biologically active compounds by replacing certain groups or bonds that contribute to the undesirable properties with bioisosteres. Bioisosteres are known to

those of skill in the art. For example the methylene bioisostere CH_2S has been used as an amide replacement in enkephalin analogs (see, e.g., Spatola (1983) pp. 267-357 in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, Weistein, Ed. volume 7, Marcel Dekker, New York). Morphine, which can be administered orally, is a compound that is a peptidomimetic of the peptide endorphin. For purposes herein, cyclic peptides are included among pepidomimetics.

As used herein, "production by recombinant means by using recombinant DNA methods" means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

As used herein, "heterologous" or "foreign" with reference to nucleic acids, cDNA, DNA and RNA are used interchangeably and refer to nucleic acid, DNA or RNA that does not occur naturally as part of the genome in which it is present or which is found in a location(s) or in an amount in the genome that differs from that in which it occurs in nature. It can be nucleic acid that has been exogenously introduced into the cell. Thus, heterologous nucleic acid is nucleic acid not normally found in the host genome in an identical context. Examples of heterologous nucleic acids include, but are not limited to, DNA that encodes a gene product or gene product(s) of interest, introduced, for example, for purposes of gene therapy or for production of an encoded protein. Other examples of heterologous DNA include, but are not limited to, DNA that encodes a selectable marker, DNA that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and DNA that encodes other types of proteins, such as antibodies.

As used herein, "expression" refers to the process by which nucleic acid, *e.g.*, DNA, is transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the nucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the mRNA.

As used herein, "vector" or "plasmid" refers to discrete elements that are used to introduce heterologous nucleic acids into cells. Typically, vectors are used to transfer heterologous nucleic acids into cells for either expression of the heterologous nucleic acid or for replication of the heterologous nucleic acid. Selection and use of such vectors

and plasmids are well within the level of skill of the art.

As used herein, "transformation" or "transfection" refers to the process by whieh nucleic acids are introduced into cells. Transfection refers to the taking up of exogenous nucleic acid, e.g., an expression vector, by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan. Successful transfection is generally recognized by detection of the presence of the heterologous nucleic acid within the transfected cell, such as, for example, any visualization of the heterologous nucleic acid or any indication of the operation of a vector within the host cell.

As used herein, "injection" refers to the microinjection (use of a small syringe) of nucleic acid into a cell.

As used herein, the amino acids, which occur in the various amino acid sequences appearing herein, are identified according to their well-known, three-letter or one-letter abbreviations (see Table 1). The nucleotides, which occur in the various DNA fragments, are designated with the standard single-letter designations used routinely in the art.

As used herein, "amino acid residue" refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferably in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in *J. Biol. Chem., 243*:3552-59 (1969) and adopted at 37 C.F.R. § § 1.821 - 1.822, abbreviations for amino acid residues are shown in Table 1:

Table 1 - Table of Correspondence

SYMBOL		
1-Letter	**3-Letter**	**AMINO ACID**
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	cysteine
X	Xaa	Unknown or other

It should be noted that all amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those referred to in 37 C.F.R. § § 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or to an amino-terminal group such as NH_2 or to a carboxyl-terminal group such as COOH.

In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al. *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p.224).

Such substitutions may be made in accordance with those set forth in TABLE 2 as follows:

Table 2

Original residue	**Conservative substitution**
Ala (A)	Gly; Ser
Arg (R)	Lys
Asn (N)	Gln; His
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; Gln; Glu

Met (M)	Leu; Tyr; Ile
Phe (F)	Met; Leu; Tyr
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Other substitutions are also permissible and may be determined empirically or in accord with known conservative substitutions.

As used herein, all assays and procedures, such as hybridization reactions and antibody-antigen reactions, unless otherwise specified, are conducted under conditions recognized by those of skill in the art as standard conditions.

As used herein: "stringency of hybridization" in determining percentage mismatch is as follows:

1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C
2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C
3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C

Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, e.g., Sambrook, E.F. Fritsch, T. Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), vol. 3, p. B.13, see, also, numerous catalogs that describe commonly used laboratory solutions). SSPE is pH 7.4 phosphate- buffered 0.18 NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by T_m, which is a function of the sodium ion concentration and temperature ($T_m = 81.5^\circ C - 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - 600/l$)), so that the only parameters in the wash conditions critical to hybrid stability are sodium ion concentration in the SSPE (or SSC) and temperature.

It is understood that equivalent stringencies can be achieved using alternative buffers, salts and temperatures. By way of example and not limitation, procedures using conditions of low stringency are as follows (see also Shilo and Weinberg, *Proc. Natl. Acad. Sci. USA*, 78:6789-6792 (1981)): Filters containing DNA are pretreated for 6

hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll®, 1% BSA, and 500 μg/ml denatured salmon sperm DNA (10X SSC is 1.5 M sodium chloride, and 0.15 M sodium citrate, adjusted to a pH of 7).

Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 40°C, and then washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which can be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

By way of example and not way of limitation, procedures using conditions of moderate stringency is provided. For example, but not limited to, procedures using such conditions of moderate stringency are as follows: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhart's solution, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 55°C, and then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency which can be used are well-known in the art. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS.

By way of example and not way of limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization

mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes before autoradiography. Other conditions of high stringency which can be used are well known in the art.

As used herein, "substantially identical to a product" means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

As used herein, "isolated" when used with reference to a composition such as an antibody or portion or fragment thereof or to a protein means that such composition is in a state that is not identical to that as it may occur in nature, if it occurs in nature. Such an isolated composition typically has been manipulated or altered from its naturally occurring state in some way by the hand of man.

As used herein, "substantially pure" means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound can, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

As used herein, "target cell" refers to a cell that contains a target molecule of interest, for example, an APP and/or Aβ peptide(s).

As used herein, "test substance" refers to a chemically defined compound (*e.g.*, organic molecules, inorganic molecules, organic/inorganic molecules, proteins, peptides, nucleic acids, oligonucleotides, lipids, polysaccharides, saccharides, or hybrids among these molecules such as glycoproteins) or mixtures of compounds (*e.g.*, a library of test compounds, natural extracts or culture supernatants) whose effect on a target of interest,

e.g., Aβ peptides and/or levels thereof in a sample, is sought to be determined by, for example, methods and assays provided herein.

As used herein, the terms "a therapeutic agent," "therapeutic regimen," "radioprotectant," "chemotherapeutic" mean conventional drugs and drug therapies, including antibodies, which are known to those skilled in the art. Radiotherapeutic agents are well known in the art.

As used herein, by "homologous" (with respect to nucleic acid and/or amino acid sequences) means about greater than or equal to 25% sequence homology, typically greater than or equal to 25%, 40%, 60%, 70%, 80%, 85%, 90% or 95% sequence homology; the precise percentage can be specified if necessary. For purposes herein the terms "homology" and "identity" are often used interchangeably, unless otherwise indicated. In general, for determination of the percentage homology or identity, sequences are aligned so that the highest order match is obtained (see, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo *et al.* (1988) *SIAM J Applied Math 48*:1073). By sequence homology, the number of conserved amino acids are determined by standard alignment algorithms programs, and are used with default gap penalties established by each supplier. Substantially homologous nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid of interest. Also contemplated are nucleic acid molecules that contain degenerate codons in place of codons in the hybridizing nucleic acid molecule.

Whether any two nucleic acid molecules have nucleotide sequences that are at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" or "homologous" can be determined using known computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson *et al.* (1988) *Proc.*

Natl. Acad. Sci. USA 85:2444 (other programs include the GCG program package (Devereux, J., *et al., Nucleic Acids Research 12(I)*:387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al., J Molec Biol 215*:403 (1990); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) *SIAM J Applied Math 48*:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs include, DNAStar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI)). Percent homology or identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program (*e.g.*, Needleman *et al.* (1970) *J. Mol. Biol.* 48:443, as revised by Smith and Waterman ((1981) *Adv. Appl. Math.* 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov *et al.* (1986) *Nucl. Acids Res.* 14:6745, as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Therefore, as used herein, the term "identity" or "homology" represents a comparison between a test and a reference polypeptide or polynucleotide. As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference nucleic acid or amino acid sequences. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide length of 100 amino acids are compared. No more than 10% (i.e., 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons can be made between a test and reference polynucleotides. Such differences can be represented as point mutations randomly

distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions. At the level of homologies or identities above about 85-90%, the result should be independent of the program and gap parameters set; such high levels of identity can be assessed readily, often without relying on software.

As used herein, "primer" refers to an oligonucleotide containing two or more deoxyribonucleotides or ribonucleotides, generally more than three, from which synthesis of a primer extension product can be initiated. Experimental conditions conducive to synthesis include the presence of nucleoside triphosphates and an agent for polymerization and extension, such as DNA polymerase, and a suitable buffer, temperature and pH.

As used herein, "animals" include any animal, such as, but are not limited to, goats, cows, deer, sheep, rodents, pigs and humans. Non-human animals, exclude humans as the contemplated animal.

As used herein, the term "subject" is used interchangeably with the term "individual" and includes mammals, such as humans.

B. Pathogenesis of Alzheimer's Disease

Neuropathologically, AD is characterized by massive neuronal cell loss in certain brain areas, and by the deposition of proteinaceous material in the brains of AD patients. These deposits are the neurofibrillary tangles and the β-amyloid plaques. The major protein component of the β-amyloid plaque is the Aβ peptide which is derived from processing of amyloid precursor protein (APP). Increased accumulation of Aβ peptide has been postulated to be a causal factor in the pathogenesis of AD. Supportive evidence for the causal role of Aβ in AD can be found in patients with Down's syndrome, who often develop AD-like symptoms and pathology after age 40. Down's syndrome patients produce elevated APP presumably due to an additional copy of chromosome 21 and exhibit AD-like amyloid plaques prior to the onset of other AD symptoms, suggesting that increased amyloid accumulation is an initial event (Giaccone G. *et al.*, (1989) *Neurosci Lett 97*:232-8). Additional evidence implicating accumulation of Aβ peptides

in AD comes from various recently identified mutations accounting for certain types of inherited AD. For example, alterations in APP processing have been linked to a subset of familial AD patients (FAD) with autosomal dominant mutations in APP (Goate, A. et al., (1991) Nature 349:704-6; Citron, M. et al., (1992) 360:672-4), presenilin 1 (Sherrington, R. et al., (1995) Nature 375:754-60), and presenilin 2 (Levy-Lehad, E., et al., (1995) Science 269:970-3). FAD individuals comprise 10% of all AD cases and generally exhibit symptoms of the disease much earlier than sporadic AD patients. For example, a double mutation of amino acids 670 and 671 of APP from Lys-Met to Asn-Lys, respectively, immediately upstream of the β-cleavage site of Aβ ("Swedish" mutation or $APP_{\Delta NL}$) results in a 5-8-fold increase in the formation of Aβ by cells (Citron, M. et al., (1992) 360:672-4). The fact that such alterations are sufficient to cause AD-like pathology is supported by studies which show that transgenic mice overexpressing $APP_{\Delta NL}$ (Hsiao, K., et al., (1996) Science 274:99-102) produce higher levels of Aβ prior to the exhibition of other AD pathological features such as abnormal phosphorylation of cytoskeletal tau, microgliosis, reactive astrocytosis, reduced levels of synaptic marker proteins and memory deficits.

1. Aβ production

Aβ peptides are derived from processing of an amyloid precursor protein (APP). Although there are several isoforms of APP, forms that contain a single-transmembrane protein have an approximately 590-680 amino acid long extracellular amino-terminal domain and an approximately 55 amino acid cytoplasmic tail which contains intracellular trafficking signals. Within APP, the Aβ peptide sequence is located partially on the extracellular side of the membrane and extends partially into the transmembrane region. Positions 29-42 on the Aβ peptide lie entirely within the putative transmembrane region and are hydrophobic in nature (Miller *et al.* (1993) *Arch. Biochem. Biophys 301*:41-52). mRNA generated from the APP gene on chromosome 21 undergoes alternative splicing to yield about 10 possible isoforms, three of which (the 695, 751, and 770 amino acid isoforms; see SEQ ID NOs: 30, 28 and 2, respectively, for exemplary amino acid sequences) predominate in the brain. APP_{695} is the shortest of the three isoforms and is produced mainly in neurons. Alternatively, APP_{751}, which contains a Kunitz-protease

inhibitor (KPI) domain, and APP_{770}, which contains both the KPI domain and an MRC-OX2 antigen domain, are found mostly in non-neuronal glial cells. All three isoforms share the same Aβ, transmembrane, and intracellular domains and are thus all potentially amyloidogenic.

APP is trafficked through the constitutive secretory pathway, where it undergoes post-translational processing including a variety of proteolytic cleavage events. APP can undergo proteolytic processing via two pathways: an amyloidogenic pathway and a non-amyloidogenic pathway. In the non-amyloidogenic pathway, cleavage of APP by α-secretase occurs within the Aβ domain releasing a large soluble N-terminal fragment (sAPPα) for secretion and a non-amyloidogenic C-terminal fragment (C83) of about 10 kD. Because α-secretase cleaves within the Aβ domain, this cleavage precludes Aβ formation. Rather, the C-terminal fragment of APP generated by α-secretase cleavage is subsequently cleaved by γ-secretase within the predicted transmembrane domain to generate a 22-24 residue non-amyloidogenic peptide fragment termed p3. Alternatively, in the amyloidogenic pathway, cleavage of APP by β-secretase (BACE) occurs at the beginning of the Aβ domain defining the amino terminus of the Aβ peptide. This cleavage generates a shorter soluble N-terminus, APPβ, as well as an amyloidogenic C-terminal fragment (C99). Further cleavage of this C-terminal fragment by γ-secretase, a presenilin-dependent enzyme, generates Aβ.

Cleavage by distinct γ-secretase activities and/or multiple γ-secretases results in C-terminal heterogeneity of Aβ, generating fragments of various lengths. For example, Aβ40 and Aβ42, which contain 40 and 42 amino acids, respectively (see, *e.g.*, SEQ ID NO: 4; amino acids 1-40 and 1-42), are thought to be cleaved by a cysteine protease and a serine protease, respectively (Figueiredo-Pereira *et al.* (1999) *J. Neurochem. 72(4)*:1417-22). Thus, selective modulation of the production of a particular form of Aβ should be possible by targeting appropriate enzymes.

The predominant forms of Aβ found in plaques are the Aβ40 and Aβ42 variants. Aβ42 accumulates primarily intracellularly, representing only 5-15% of the total Aβ secreted by most cell lines (Wang, *et al.*, (2001) *Neurobiology of Aging 23*:213-223). Published immunohistochemical studies have demonstrated that in brains of individuals

harboring FAD-linked mutations in APP (Val to Ile at codon 717), Aβ42 is deposited early and selectively in the cerebral cortex. This holds true in numerous studies with transgenic mice and in FAD patients harboring mutations in presenilin genes known to increase Aβ42 formation (relative to Aβ40). In the AD cerebral cortex, virtually all AD plaques are Aβ42 immunopositive while only approximately one third are Aβ40 immunopositive. In fact, diffuse amyloid plaques, representing the earliest stage of amyloid deposition, are almost exclusively composed of Aβ42 (Iwatsubo *et al.* (1994) *Neuron 13*: 45053; Borchelt *et al.* (1997) *Neuron 19*: 939). In vitro experiments have demonstrated that Aβ42 polymerizes faster than Aβ40, suggesting that the carboxy terminus of Aβ determines the aggregation potential, and therefore, is one of the critical determinants for the rate of amyloid fibril formation (Parvathy, *et al.*, (2001) *Arch Neurol. 58*: 2025-2032). Aβ42 has also been shown to dramatically enhance precipitation of Aβ40 in vitro. Therefore, the Aβ42 species of amyloid peptide is a primary target in the development of therapeutics for the treatment of neurodegenerative disease characterized by Aβ plaque formation. Aβ42 accumulation predominantly affects neurons in the cerebral cortex and hippocampus of AD brains prior to the appearance of amyloid plaques. Neurons burdened with excessive Aβ42 can lose function and eventually undergo lysis, resulting in local dispersal of their cytoplasmic contents.

Production of Aβ can occur at several distinct locations along the secretory pathway. APP produced in the endoplasmic reticulum (ER) transits to the Golgi, where it is post-translationally modified via N- and O-linked glycosylation and tyrosine sulfation before vesicular transport to the cell surface. Cell surface APP is then reinternalized via endocytosis into the endosomal/lysosomal system where it may be degraded. Cleavage of APP to form Aβ can occur in at least three sites along this pathway. The endosomal-lysosomal system may contribute minor amounts of secreted Aβ, particularly in non-neuronal cells. The trans-Golgi network (TGN) is the major site of intracellular Aβ40 production in neurons and in non-neuronal cells transfected with mutant APP. In addition, either the TGN or post-Golgi vesicles are responsible for the production of secreted Aβ in neurons. Finally, the ER is a site for the production of Aβ42. Aβ42 produced in the ER is found in an intracellular stable insoluble pool. The

proteosome may aid in the degradation of these ER-generated APP fragments (Skovronsky (2000) *Biochemistry 39(4)*:810-7). Due to the organelle-specific differences in the generation and clearance/degradation of Aβ peptides, it is possible to selectively modulate the production, clearance and/or degradation of a particular form of Aβ by targeting appropriate γ-secretases and/or degradative enzymes.

Presenilins, multitransmembrane proteins localized predominantly to the ER and Golgi, play a crucial role in APP processing. Presenilin-1 (PS-1) was first identified as an early onset gene in Alzheimer's disease and is believed to be a critical component of the enzyme complex which cleaves the amyloid precursor protein (APP) at the γ-secretase site to produce Aβ. Over 40 dominant point mutations in PS-1 (chromosome 14) and PS-2 (chromosome 1) as well as one splice site mutation in PS-1 have been associated with familial AD (FAD) phenotypes (see, *e.g.*, Van Gassen *et al.* (2000) *Neurobiol. Dis.* 7:135-151; Hardy (1997) *Trends Neurosci. 20*:154-159; and Cruts and Van Broeckhoven (1998) *Ann. Med. 30*:560-565). Thus, presenilins are involved in the carboxy-terminal cleavage of APP in both normal and pathological states. Involvement of presenilin has also been shown in the cleavage of additional membrane proteins such as Notch, Erb-B4 (Lee *et al.* 2002, *J. Biol. Chem. 277(8)*:6318-23), and E-cadherin (Marambaud *et al.* 2002, *EMBO J. 21(8)*:1948-56). Presenilins may play a general role in intramembrane cleavage and, thus, may likely have additional substrates yet to be reported.

2. Aβ degradation/clearance

The accumulation of Aβ42 in the brain clearly depends on the production levels of the amyloid peptide, however numerous other factors also contribute significantly to brain Aβ42 levels. Some of these factors are Aβ42 proteolytic degradation, receptor-mediated clearance, non-receptor-mediated clearance, and/or aggregation/fibrillogenesis. Therefore, defects in pathways for Aβ degradation and clearance could underlie some or many cases of familial and sporadic AD as well as other diseases and disorders characterized by misregulation of Aβ. Understanding how Aβ degradation and clearance is regulated in the cerebral cortex has implications for both the pathogenesis and the treatment of such diseases and disorders. Agents that affect any of these

pathways/mechanisms can be useful as therapeutic drugs.

Metabolic labeling studies in living mice show that newly generated Aβ is very rapidly turned over in the brain (Savage *et al.*, (1998), *J. Neurosci 18*:1743-1752), suggesting that Aβ-degradation proteases help regulate its levels. There are numerous proteases in the brain that could potentially participate in Aβ turnover, and there is evidence that several enzymes may contribute to the degradation of Aβ peptides in brain tissue including insulin-degrading enzyme (IDE), neprilysin, plasmin, uPA/tPA, endothelin converting enzyme-1, and matrix metalloproteinase-9 (Selkoe J. (2001) *Neuron 32*:177-180). IDE has been shown to degrade insulin, glucagon, atrial naturetic peptide, calcitonin, TGF-α, and amylin, among other small peptides of diverse sequence. IDE is believed to have little dependence on sequence specificity but recognizes a conformation that is prone to conversion to a β-pleated sheet structure. Such a property is concurrent with its propensity to degrade several peptides that undergo concentration dependent formation of amyloid fibrils (e.g., insulin, ANF, amylin, calcitonin, and Aβ). It believed that the motif recognized by IDE is not the β-pleated sheet region per se but a conformation of the monomer in a pre-amyloid state. IDE occurs principally in a soluble form in the cytoplasm. However, a form of IDE can be labeled on the cell surface, including in neurons, and is also present on intracellular membranes (Vekrellis *et al.* (2000) *J. Neurosci. 20*: 1657-1665). The existence of a membrane-anchored form of the protease suggests that it could help regulate insulin signaling at the plasma membrane and could also participate in the degradation of both soluble and membrane-associated forms of Aβ.

Neprilysin is a member of the neutral endopeptidase family of membrane-anchored proteases found on the cell surface. Neprilysin has been implicated in the degradation of Aβ peptides (Iwata *et al.*, (2000) *Nat. Med. 6*:143-150; Carson and Turner, (2002) *J. Neurochem 81(1)*: 1-8), mediating the degradation of predominantly insoluble forms of Aβ. In addition, it has been shown that steady state levels of endogenous Aβ are elevated in the brains of young neprilysin-deficient mice (Iwata *et al.*(2001) Science 292: 1550-1552). The rise, while highly significant, was not large, and plaque formation was not observed. Thus, it is believed that other proteases, including

additional members of the neutral endopeptidase family, may function to degrade Aβ.

The plasmin proteolytic cascade, known to be crucial for fibrinolysis and cell migration, has been implicated in Aβ clearance as well. In this cascade, either of two activators of plasmin, tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA), can be post-translationally activated by binding to fibrin and other substrates. In vitro studies have suggested that Aβ aggregates can substitute for fibrin aggregates in activating tPA. In the nervous system, plasminogen, tPA and uPA are expressed in neurons, and tPA is also synthesized by microglia. In vitro assays have indicated that pure plasmin can proteolyze monomeric Aβ and fibrillar Aβ at a considerably lower efficiency.

Another protease expressed in brain that has been evaluated for its ability to degrade Aβ is endothelin converting enzyme-1 (ECE-1) (Eckman *et al.* (2001) *J. Biol. Chem. 276*: 24540-24548). This integral membrane zinc metalloprotease, with its active site located in the lumen and extracellularly, can cleave the endothelin precursors and several other biologically active peptides, including bradykinin, substance P, and the oxidized insulin B chain. Cellular overexpression of ECE-1 leads to a marked reduction in the levels of naturally secreted Aβ40 and Aβ42 peptides in Chinese hamster ovary cells. The purified enzyme directly proteolyzed both synthetic peptides in vitro. Other purified proteases that have been reported to digest synthetic Aβ peptides under in vitro conditions include matrix metalloproteinase-9 and cathepsin D.

In addition, several cell surface receptors have been implicated in Aβ clearance, including the scavenger receptor A (Paresce *et al.*, (1996) *Neuron 17*:553-565), the receptor for advanced glycation endproducts (RAGE) (Yan et al., (1996) *Nature 382*: 685-691), and the low-density lipoprotein receptor-related protein-1 and -2 (LRP-1 and LRP-2) (Narita *et al.*, (1997) *J. Neurochem. 69*:1904-1911; Shibata *et al.*, (2000) *J. Clin. Invest. 106(12)*: 1489-99; Kang *et al.*, (2000) *J. Clin. Invest. 106(9)*: 1159-66; Ulery and Strickland, (2000) *J. Clin. Invest.* 106(15): 1077-9; Hammad *et al.*, (1997) *J. Biol. Chem.* 272(30): 18644-9). Scavenger receptor binding to Aβ has been shown to facilitate the uptake of Aβ by microglia. Microglia are immune system cells associated with Alzheimer's disease plaques containing Aβ. These cells facilitate phagocytosis of

amyloid fibrils into the endosomal/lysosomal system where they may subsequently be degraded by acid hydrolases in late endosomes and lysosomes (Selkoe (2001) *Neuron 32*: 177-180). The scavenger receptors expressed by microglia appear to play a significant role in this clearance process and, thus may be useful targets for the identification of agents that modulate Aβ levels.

Binding of Aβ to neuronal RAGE induces activation of nuclear factor κB (NF-κB), which drives expression of macrophage-colony stimulating factor (M-CSF). M-CSF signals microglia from distant sites, drawing them toward loci of neuronal perturbation and inducing cell activation, including increased proliferation, and enhanced expression of microglial scavenger receptors and apoE. Such activation may lead to increased clearance of Aβ through microglial phagocytic pathways.

LRP is a multifunctional receptor with four distinct ligand binding domains and at least 14 identified ligands, including apolipoprotein E (apoE), apoJ, α2-macroglobulin (α2M), and lactoferrin. LRP is involved in receptor-mediated endocytosis, directing ligands to degradation via the late endosome and lysosome. Aβ has been found to bind several LRP ligands including apoE (Holtzman, (2001) *J. Mol. Neurosci. 17(2)*:147-55), apoJ (Hammad *et al.*, (1997) *J. Biol. Chem.* 272(30): 18644-9), and activated α2M (α2M*) (Qiu *et al.*, (1999) *J. Neurochem 73(4)*:1393-8). Such ligand interactions, and specifically the binding of Aβ to α2M*, are believed to facilitate Aβ clearance through an LRP-mediated endocytic pathway. Identification of agents which modulate LRP and/or components of LRP-mediated clearance pathways provides an attractive approach for therapeutic intervention.

The proteosome has also been implicated in the degradation of ER-generated APP fragments, specifically Aβ42 (Skovronsky (2000) *Biochemistry 39(4)*:810-7). General phagocytic mechanisms and up-regulation of genes in response to inflammatory stimuli are also reported to enhance Aβ clearance. In addition, metal chelators, such as clioquinol (Cherny *et al.* (2001) *Neuron 30*:655-61), are believed to play a role in dissolving plaques and/or preventing Aβ aggregation.

3. Reduction of Aβ accumulation

Based on the strong correlation between Aβ accumulation, neuronal loss and AD,

a reduction in Aβ accumulation should result in decreased plaque formation and minimize neuronal cell death. There are, however, numerous mechanisms and activities which may influence brain Aβ levels, and these mechanisms can influence many other important cellular functions and processes. For example, production of an intracellular C-terminal fragment (CTF) of APP resulting from γ-secretase cleavage between amino acids 49 and 50, close to the cytoplasmic side of the transmembrane domain, is believed to play a role in signal transduction (Pinnix, I *et al.* (2001) *J. Biol. Chem 276*:481-487; Sastre, M. *et al.* (2001) *EMBO Reports 2(9)*:835-41; Gu, Y *et al.* (2001) *J. Biol Chem. 276(38)*: 35235-8; Cao, X and Sudhof, T.C. (2001) *Science 293*:115-120). Inhibition of such cleavage may result in unwanted side affects. It is, therefore, important when seeking agents for altering Aβ levels to identify agents that act specifically on the Aβ endpoint with minimal disruption of other, often overlapping, cellular pathways and processes. Due to the high degree of regulation of and organelle-specific differences in the generation, clearance, and degradation of the various Aβ peptides, identification of agents that target appropriate production enzymes, degradative enzymes, and/or related proteins and receptors involved in Aβ production and clearance pathways should make possible modulation of the production, clearance and/or degradation of one or more Aβ peptides without substantially affecting other cellular compositions, processes and activities.

One approach to treating diseases associated with Aβ-based amyloidosis, such as Alzheimer's disease, is aimed at reducing Aβ peptide production by targeting presenilin function. However, because presenilin and presenilin-dependent activities affect substrates other than APP, non-specific modulation (such as, for example, inhibition) of presenilin and/or presenilin-dependent mechanisms can result in unwanted side effects. Furthermore, because γ-secretase generates normal non-amyloidogenic peptides, such as p3 and APP CTF, non-specific modulation of γ-secretase may be undesirable. In addition, because release of Aβ peptides is a normal event in virtually every cell, it may be desirable in some instances to maintain or even elevate levels of particular Aβ peptides.

There is a need for agents that modulate the levels of one or more Aβ peptides of

cells and tissues (intracellular, extracellular, and/or membrane-bound Aβ), for example, by modulating compositions (*e.g.*, proteases and proteins, such as proteins on which protease activities depend, including presenilins), mechanisms and/or activities involved in Aβ peptide formation and persistence in cells and/or extracellular medium without substantially affecting (or with only limited or minimal effect on) compositions, mechanisms and/or activities that are not significantly involved in Aβ peptide formation and persistence. There is particularly a need for agents that modulate the levels of Aβ42 peptide in cells and/or extracellular medium without substantially affecting (or with only limited or minimal effect on) compositions, mechanisms, processes and/or activities that are not significantly involved in Aβ42 peptide generation and persistence in cells and/or extracellular medium. Such agents have numerous uses. For example, such agents can be used in elucidating the precise elements and pathways involved in Aβ peptide formation, degradation and clearance in cells. Furthermore, such agents are candidates for the prevention and/or treatment of diseases and disorders involving amyloidosis, such as, for example, AD. Such agents can provide therapeutic and/or prophylactic benefit with limited-to-no potential side effects that can result from non-specific modulation of Aβ peptide processing and/or clearance.

Provided herein are methods of identifying agents that modulate the levels (including, *e.g.*, cellular and/or extracellular) of one or more Aβ peptides. In particular embodiments, the methods can be used to identify agents that modulate the levels of Aβ42 (including cellular and/or extracellular). In further embodiments, the methods can be used to identity agents that selectively modulate the levels of Aβ42 (including cellular and/or extracellular).

In another embodiment, the methods can be used to identify agents that modulate Aβ peptide levels (and, in particular, Aβ42 levels) without substantially affecting (or with limited, minimal or inconsequential effect on) compositions, mechanisms, processes and/or activities that are not significantly involved in the generation, degradation and/or clearance of one or more Aβ peptides. A composition, mechanism, process or activity that is not significantly involved in the generation, degradation and/or clearance of an Aβ peptide can be, for example, one that has minimal effect on the generation, degradation

and/or clearance of an Aβ peptide. Thus, for example, if the generation, degradation and/or clearance of an Aβ peptide does not differ substantially in the presence and absence of a particular composition, mechanism, process or activity, then the composition, mechanism, process or activity may not be significantly involved in the generation, degradation and/or clearance of an Aβ peptide. In a particular embodiment, the method involves a step of identifying an agent that modulates the levels (including *e.g.*, cellular and/or extracellular) of one or more Aβ peptides without substantially altering the substrate-processing activity of presenilin. The method can involve a step of identifying an agent that modulates the levels of one or more Aβ peptides without substantially altering the cleavage of a presenilin substrate, or portion(s) thereof, that is other than APP. In a further embodiment, the presenilin substrate is LRP. In another embodiment, the method involves a step of identifying an agent that modulates the levels (including, *e.g.*, cellular and/or extracellular) of one or two Aβ peptides, without substantially altering the levels of one or more other Aβ peptides. In a particular embodiment, an agent that modulates the levels of Aβ42 only, or Aβ39 only, or Aβ42 and Aβ39 only, without substantially altering the levels of one or more other Aβ peptides, is identified. The agent can be, for example, one that modulates the levels of Aβ42 and/or Aβ39 without substantially altering the levels of Aβ40.

Also provided herein are methods of modulating Aβ peptide levels (including, *e.g.*, cellular and/or extracellular Aβ). In one embodiment, the method includes a step of contacting a sample, for example, a cell, with an agent that modulates the level of one or more Aβ peptides, in particular, Aβ42 and/or Aβ39, without substantially affecting or altering the level of one or more different Aβ peptides. For example, the method can include a step of contacting a sample, for example, a cell, with an agent that modulates Aβ42 and/or Aβ39 levels without substantially altering the levels of Aβ40. In another embodiment, the methods include a step of contacting a sample (*e.g.*, a cell) with an agent that modulates the level of one or more Aβ peptides, particularly Aβ42, without substantially affecting a non-APP substrate-processing activity of presenilin. The methods can include a step of contacting a sample with an agent that modulates the level of one or more Aβ peptides without substantially affecting the cleavage and/or

processing of a presenilin substrate other than APP. In a particular embodiment, the presenilin substrate is LRP.

Further provided herein is an antibody that selectively recognizes Aβ42 without substantially binding to other Aβ peptides. The antibody has numerous uses and provides specific advantages as compared to other antibodies. For example, the antibody can be used in methods of identifying agents that modulate Aβ42 levels without substantially affecting the level of other Aβ peptides. The antibody can further be used in methods of detecting Aβ42 in a sample for any purpose, including but not limited to methods of diagnosis of diseases and disorders involving amyloidosis, for example, AD.

Also provided herein are compositions and methods for assessing presenilin activity and/or presenilin-dependent activity. In one embodiment, the methods involved determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP in a sample for which presenilin activity is being assessed. The methods can be used in methods for identifying or screening for agents that modulate presenilin and/or presenilin-dependent activity that are also provided herein. As described herein, presenilins are proteins that are involved in the processing of a number of proteins with various functions and activities, including not only APP but LRP. Because presenilins are involved in diverse reactions with a variety of substrates, it is desirable to identify agents that affect presenilin activity and presenilin-dependent mechanisms. A method provided herein for identifying agents that modulate presenilin and/or presenilin-dependent mechanisms is based on the finding described herein that LRP is a substrate that is processed in a presenilin-dependent mechanism. In one embodiment, the method includes a step of comparing the levels and/or composition of LRP C-terminal fragments in samples containing presenilin that have been contacted with a test agent and samples containing presenilin that have not been contacted with test agent. The methods for identifying an agent that modulates presenilin activity can be applied to methods for identifying candidate agents for the treatment or prophylaxis of a disease or disorder associated with altered presenilin. One embodiment of these methods includes steps of contacting a sample containing LRP and an altered presenilin that is associated with altered LRP processing with a test agent and identifying a candidate

agent that restores LRP processing to that which occurs in the presence of a presenilin that is not associated with altered processing of LRP.

C. Methods of Assessing Presenilin and/or Presenilin-Dependent Activity

Presenilins are transmembrane proteins localized predominantly in the ER and Golgi. Included among the presenilin proteins are the homologous presenilin-1 (PS1) and presenilin-2 (PS2) proteins (see SEQ ID NO: 6 for an amino acid sequence of a PS1 protein and SEQ ID NO: 8 for an amino acid sequence of a PS2 protein). Although the presenilin proteins alone may not have an enzymatic activity, they appear to play an essential role in the proteolytic processing of a variety of proteins, including APP (particularly the γ-secretase cleavage of APP) and in the trafficking and maturation of various cellular proteins (referred to herein collectively as substrates for presenilin activity and/or presenilin-dependent enzyme activity), including, but not limited to Notch, TrkB, APLP2, hIre1α, E-cadherin and Erb-B4. With respect to processing of APP, it appears that presenilin participates intimately as part of a catalytic complex by which γ-secretase mediates an intramembranous proteolysis of APP. Two transmembrane aspartate residues (D257 and D385 in PS1; D263 and D366 in PS2) are individually critical for presenilin-associated γ-secretase activity as well as presenilin endoproteolysis.

Inherited mutations in the genes encoding presenilins-1 and -2 account for up to 40% of the early onset cases of familial Alzheimer's Disease (FAD). FAD-associated mutations in PS1 and PS2 give rise to an increased accumulation of Aβ42 in AD patients and transfected cell lines and transgenic animals expressing FAD mutant forms of PS1 or PS2.

Because presenilins and presenilin-dependent activities play a key, yet mechanistically unresolved, role in the cleavage of numerous proteins involved in a variety of processes (some of which are associated with diseases such as Alzheimer's Disease), there is a need for compositions and methods that can be used in assessing presenilin activity. For example, assessment of presenilin activity using such compositions and methods can greatly facilitate the elucidation of the mechanisms of protein processing in normal and disease states and the determination of the number,

specificities, regulation and potential overlap of the proteolytic activities that function in the cleavage of an array of transmembrane proteins. Furthermore, compositions and methods for the assessment of presenilin activity may also be used in screening of agents that specifically modulate various presenilin-dependent enzyme activities. Such agents may also be of use in elucidating the mechanisms of protein processing in normal and disease states. In addition, such agents can be candidate agents for the prevention and/or treatment of diseases associated with altered proteolytic processing of cellular proteins, such as, for example, diseases involving amyloidosis, including AD.

Provided herein are compositions and methods for assessing presenilin activity and/or presenilin-dependent activity. In one embodiment, the methods involve determining the level of one or more fragments of LRP and/or the composition of LRP in the presence of a sample for which presenilin activity is being assessed. Determining the level can be determining the presence or absence of one or more fragments as well as making a more quantitative assessment of amount of the fragment. The methods are based on the finding described and demonstrated herein that the low density lipoprotein receptor-related protein (LRP) is processed by a presenilin-dependent enzyme activity. In a particular method for assessing presenilin activity, the level of a fragment of LRP that is from a C-terminal portion of LRP is determined, such as, for example, an approximately 20 kD-fragment. Also provided is a method of identifying agents that modulate presenilin activity and/or presenilin-dependent activity which involve comparing, in the presence and absence of test agents, the level of one or more fragments of LRP and/or composition of LRP in the presence of a presenilin activity. In a particular embodiment, the level of a fragment of LRP that is from a C-terminal portion of LRP is determined, such as, for example, an approximately 20 kD-fragment. Determining the level for any of these methods can be determining the presence or absence of one or more fragments as well as making a more quantitative assessment of amount of the fragment.

Further provided is a method for identifying candidate agents for the treatment and/or prevention of a disease or disorder, such as a disease or disorder associated with altered presenilin function or activity, which includes a step of comparing, in the presence and absence of test agents, the level of one or more fragments of LRP and/or

composition of LRP in the presence of a presenilin encoded by a mutant or polymorphic nucleic acid. In a particular embodiment, the level of a fragment of LRP that is from a C-terminal portion of LRP is determined, such as, for example, an approximately 20 kD-fragment. In particular embodiments, the disease or disorder is associated with amyloidosis, for example, Alzheimer's disease. The mutant nucleic acid can be, for example, one that encodes a presenilin that is linked to Alzheimer's disease. For example, the mutant nucleic acid may encode any one or more of the at least 60 mutations in human PS1 and the at least two mutations in human PS2 that have been genetically linked to early onset familial Alzheimer's disease (FAD) (see, *e.g.*, Van Gassen *et al.* (2000) *Neurobiol. Dis.* *7*:135-151; Checler (1999) *IUBMB Life* *48*:33-39; St. George-Hyslop (2000) *Biol. Psychiatry* *47*:183-199; Steiner *et al.* (1999) *Eur. Arch. Psychiatry Clin. Neurosci.* *249*:266-270). Included among such mutations are the PS2 FAD mutation N141I (Volga German FAD mutant) and the PS1 FAD mutation M146L.

Additional methods of assessing presenilin activity and/or presenilin-dependent enzyme activity involve determining the levels and/or compositions of fragments of other presenilin substrates. Presenilin substrates are peptides, polypeptides, proteins or fragments thereof that are proteolytically processed, at least in part, in a presenilin-dependent manner. Thus, if presenilin is absent, or presenilin activity is inhibited or reduced, the proteolytic processing of a presenilin substrate is altered, for example by an alteration in the levels and/or composition of fragments generated from the substrate, relative to the proteolytic processing of the substrate that occurs in the presence of normal (*e.g.*, wild-type) presenilin activity. Generally, a presenilin substrate can contain about one transmembrane domain, an ectodomain that is released or shed into the extracellular medium, and/or an intracellular domain. Typically, processing of a presenilin substrate includes an initial cleavage of the substrate (typically by a metalloprotease) at a site located in the extracellular domain of the substrate to release an ectodomain of the substrate, followed by presenilin-mediated cleavage of the remaining membrane-bound portion of the substrate to yield an intracellular fragment, which may be translocated to the nucleus of a cell.

1. LRP Assay

a. LRP

Low density lipoprotein receptor-related protein (LRP) is a cell surface receptor that binds and internalizes a number of diverse extracellular ligands, including apolipoprotein E (apoE), α2-macroglobulin (α2M), APP, tissue-type plasminogen activator (tPA) and lactoferrin, for degradation by lysosomes. LRP expression is widespread; however, it is most highly expressed in the liver, brain and placenta. With respect to its expression in the brain, LRP is primarily a neuronal receptor expressed in the cortex and hippocampus and is also expressed in activated astrocytes, glia and microglia. Mature LRP is a heterodimer containing an N-terminal 515 kD extracellular subunit (α chain) and a C-terminal 85 kD membrane-anchored subunit (β chain) which are non-covalently associated. The mature receptor is generated by proteolytic cleavage of a 600 kD precursor polypeptide in a trans-Golgi compartment in a process that involves the endoproteinase furin. The amino acid sequence of the LRP precursor polypeptide is provided in SEQ ID NO: 10 (see also GenBank Accession No. Q07954), and DNA encoding the polypeptide is provided in SEQ ID NO: 9. Proteolytic processing of precursor LRP to yield the mature receptor occurs at amino acid position 3925 C-terminal to the tetrabasic amino acid sequence RHRR. LRP is anchored in the plasma membrane by a single transmembrane domain, and its cytoplasmic tail includes two copies of the internalization signal NPXY.

Additionally, LRP undergoes another proteolytic processing step at the cell surface which involves a metalloproteinase (Quinn *et al.* (1999) *Exp. Cell. Res. 251*:433-441). This processing results in "shedding" from the cell surface of a portion of LRP containing the α chain (an ~500-kD soluble polypeptide) that is noncovalently associated with a truncated β chain (the extracellular portion of the β chain (i.e., amino acids 3944-4420 of SEQ ID NO: 10; Mr =~67 kD or Mr =~55 kD after deglycosylation with *N*-glycosidase F).

LRP is a member of the low-density lipoprotein receptor (LDLR) family. The extracellular region of receptors in this family contains several structural modules which include ligand-binding repeats of ~40 amino acids (including six cysteine residues forming three disulfide bonds), epidermal growth factor (EGF) precursor repeats (each

also containing six cysteine residues), and modules with a consensus tetrapeptide (YWTD). In addition to these modules, these receptors contain a single transmembrane domain and a relatively short cytoplasmic tail with endocytosis signals and elements for interaction with cytoplasmic adaptor and scaffold proteins (*e.g.*, Dab, FE65, c-jun N-terminal kinase interacting proteins (JIPs) and postsynaptic density protein PSD-95) for mediating signal transduction.

LRP may have a significant role in the pathogenesis of AD. Several LRP ligands, including apoE, lactoferrin and α2M, bind Aβ. Such ligand interactions are believed to facilitate Aβ clearance through an LRP-mediated endocytic pathway (Qiu *et al.* (1999) *J. Neurochem. 73*:1393-8). LRP levels are reduced in AD and in transgenic mice expressing presenilin and cells transfected with presenilin-encoding DNA. Furthermore, transgenic mice overexpressing the M146L or L286V presenilin-1 mutations associated with AD reportedly have decreased levels of LRP expression in certain neuronal populations. LRP also interacts with APP via adaptor proteins, such as FE65. In addition, genetic association studies indicate that the LRP gene may be a susceptibility locus for late-onset AD.

b. LRP is processed by a presenilin-dependent activity

As described and demonstrated herein (see the EXAMPLES), LRP is processed by a presenilin-dependent enzyme activity. LRP processing was analyzed in cell lines expressing defective (i.e., loss of function) PS1 proteins encoded by nucleic acid lacking exons 1 and 2 (see, *e.g.*, GenBank Accession No. L76518 for sequences of exons 1 and 2) of the PS1-encoding DNA or nucleic acid coding for an alanine instead of an aspartic acid residue at amino acid 385 (D385A), which is essential to PS1 function. These cells had been generated by transfecting mouse neuroblastoma (N2a) cells (see, *e.g.*, ATCC, Rockville, MD), which express endogenous LRP, with nucleic acid encoding wild-type human APP695 and nucleic acid encoding human PS-1 (wild-type, D385A mutant, or exon 1 and 2 deletion). It was discovered that LRP processing is altered in the cells expressing defective PS1 proteins relative to cell lines expressing normal wild-type PS1. Specifically, an ~20-kD peptide was detected in an immunoassay of lysates of cells that had been transfected with mutant PS1-encoding DNA that was not detected (or detected

at much lower levels) in lysates of cells that had been transfected with wild-type PS1-encoding DNA. The detection antibody (R9377) was one generated against the carboxyl-terminal 13 amino acids of human LRP. Because the ~20-kD peptide from a C-terminal portion of LRP, which contains an epitope recognized by an antibody generated against the C-terminal 13-amino acids of LRP, was absent or only barely detectable in lysates of cells expressing a wild-type PS1, but present at readily detectable levels in lysates of cells that contain mutant PS1 protein, it appears that a PS1-dependent activity cleaves LRP in such a way as to eliminate an amino acid sequence in a C-terminal region of LRP that is recognized by C-terminus-reactive antibody. The processing of APP and Notch, two substrates for presenilin-dependent processing activity, was also analyzed in these cells, in addition to the analysis of LRP processing. Analogous results, in which particular C-terminal fragments of APP and Notch were detected in lysates of PS1 mutant cells but not in lysates of wild-type PS1 cells, were obtained in analyses of APP and Notch processing. Thus, the results revealed a concordance of the activity of PS-1 with the three substrates. The similar findings support a conclusion of a presenilin-dependent cleavage of LRP. It was also found that the LRP β chain alone is sufficient for processing by PS-1, and that trafficking to the plasma membrane is a necessary event for the normal processing of LRP by the PS-1 active complex.

As also described in the EXAMPLES, in the presence of the γ-secretase inhibitor DAPT (N-[N-(3,5,-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine-*t*-butyl ester), an accumulation of an approximately 20 kD fragment of LRP that is from a C-terminus portion of LRP is observed. The fragment is one that is recognized and bound by a polyclonal antibody (*e.g.*, antibody R9377 as described in the EXAMPLES) generated against a carboxyl-terminal peptide (the carboxyl-terminal 13 amino acids) of human LRP (C-GRGPEDEIGDPLA) with N-terminal cysteine added for conjugation to ovalbumin. The accumulation of the ~20-kD fragment from a C-terminal portion of LRP parallels the accumulation of APP C-terminal fragments (CTFs). This finding indicates that LRP fragment accumulation is a measure of presenilin/γ-secretase activity. Advantages of using LRP fragment analysis in a method for assessing presenilin activity include: (1) LRP is highly expressed in adult brain, (2) the analysis is easily amenable to

testing *in vivo* samples, (3) endogenous LRP is expressed at sufficient levels in cell culture models such that transfection the cells with nucleic acid encoding LRP in order to increase expression levels for detection is not necessary, and (4) LRP appears to have a significant role in the pathogenesis of AD. Furthermore, LRP has been shown to have a potentially significant role in the clearance of Aβ as described above.

c. Methods of modulating LRP

Because of the involvement of LRP in critical cellular processes, including, but not limited to, signal transduction and receptor-mediated endocytosis, and in mechanisms associated with Alzheimer's disease, there is a need for methods and compositions that can be used in modulating LRP. LRP modulation can be any alteration of LRP, including, but not limited to, any alteration in the processing, structure, function (including, for example ligand-binding) and/or activity (including, for example, signal transduction and receptor-mediated endocytosis) of LRP. Modulation of LRP has numerous uses. For example, the ability to modulate LRP can greatly facilitate the elucidation and detailed characterization of the mechanisms involved in signal transduction and receptor-mediated endocytosis. Furthermore, modulation of LRP has applications in the treatment and prophylaxis of diseases of signal transduction and endocytosis, as well as AD.

As described and demonstrated herein, LRP is processed by a presenilin-dependent enzyme activity. The processing of LRP can have significant effects on its structure, function and activity.

Provided herein are methods for modulating LRP. The LRP can be in a sample that has been selected for LRP modulation. Such samples include, but are not limited to, cells, tissues, organisms, lysates, extracts and membrane preparations of cells and cell-free samples containing LRP, including, for example, extracellular medium, tissue and body fluids. In one embodiment, the methods involve altering the structure, function and/or activity of a presenilin (and/or fragments thereof) in a sample containing LRP, and/or fragment(s) thereof, and a presenilin, and/or fragment(s) thereof, whereby the LRP is modulated. The structure, function and/or activity of a presenilin can be altered in a number of ways which can vary depending in large part on the sample. For example, the

function and activity of presenilins (particularly functions and activities relating to interaction of presenilin with other molecules) can be altered by contacting presenilin with antibodies, and/or fragment(s) thereof, that bind presenilin, particularly antibodies that bind to presenilin in such a way as to impede or eliminate the ability of presenilin to interact with binding partners. If the sample is a cell, the function and/or activity of presenilin in the cell can be altered, for example, by enhancing, increasing, reducing or eliminating the expression of the presenilin. Methods are known in the art for transferring nucleic acids encoding presenilin into cells and for reducing or eliminating the expression of functional proteins, such as presenilin, in cells (*e.g.*, gene knock-out, antisense RNA and RNA interference techniques).

In another embodiment, the methods involve contacting a sample containing an LRP, and/or fragment(s) thereof, and presenilin, and/or fragment(s) thereof, with an agent that modulates presenilin or presenilin-dependent activities. The sample is one that has been selected for LRP modulation. An agent that modulates presenilin or presenilin-dependent activities can be identified using methods provided and described herein.

d. Assessment of presenilin activity based on LRP

In a method for assessing presenilin activity provided herein, the level of one or more fragments of LRP and/or the composition of LRP is determined for a sample for which presenilin activity is being assessed. Examples of a sample for which presenilin activity is being assessed include, but are not limited to, a cell that expresses presenilin, a lysate or extract of a cell that expresses presenilin, or membranes prepared from a cell that expresses presenilin. The cell can endogenously express presenilin and/or express heterologous presenilin. LRP can be added to the sample or can be expressed endogenously and/or heterologously by the cell. In a particular embodiment, the method includes assessing presenilin activity of a cell by evaluating the level (which includes determining the presence or absence of) of a fragment from a C-terminal portion of LRP in a cell lysate.

To assess presenilin activity in these methods, the processing of LRP is evaluated. In evaluating LRP processing, the composition of LRP can be evaluated. The composition of LRP refers to the make-up of any LRP that is present anywhere in the

analysis mixture, including the assay medium in which the analysis is being performed, an extracellular medium, or a cell membrane, lysate or extract. Thus, in one embodiment, the structure of any LRP present can be evaluated to, for example, determine whether LRP is intact or has been processed and appears as a fragment or fragments of sizes smaller than the intact LRP molecule or than either one or both of the intact chains of LRP. In evaluating LRP processing, the levels (including the presence or absence) of one or more LRP fragments can be determined.

In particular, the LRP composition is evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage or altered presenilin-dependent cleavage of LRP are present and/or the level of any such fragment(s). A presenilin-dependent cleavage described herein occurs within the C-terminal portion of LRP and within the β chain. Thus, a presenilin-dependent cleavage of LRP can be one that occurs in the C-terminal portion of LRP at a position C-terminal to amino acid position 3925 of SEQ ID NO: 10 (or of the amino acid sequence provided as GenBank Accession No. Q07954). The presenilin-dependent cleavage of LRP can be one that occurs within the sequence of the last approximately 580, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 25, or less amino acids of LRP. The presenilin-dependent cleavage can be one that occurs C-terminal to the extracellular portion of the β chain (i.e., approximately amino acids 3944-4420 of SEQ ID NO: 10 or of the amino acid sequence provided as GenBank Accession No. Q07954); thus, C-terminal to about amino acid 4420 of SEQ ID NO: 10. The presenilin-dependent cleavage of LRP can be one that occurs near or within the region of the LRP protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of LRP can be one that generates a soluble intracellular peptide, containing the extreme C-terminus of LRP, and a membrane-associated peptide containing amino acid sequence of the transmembrane region of LRP, particularly the more C-terminal region of the transmembrane segment of LRP. Any LRP fragments generated by such presenilin-dependent activities have a molecular weight that is less than that of the β chain of LRP (β chain molecular weight is approximately 85-90 kD, or approximately 67 kD after deglycosylation with *N*-glycosidase F). In particular

embodiments, an LRP fragment generated by such presenilin-dependent activities has a molecular weight that is less than that of the extracellular portion of the β chain of LRP (the extracellular portion of the β chain molecular weight is approximately 67 kD, or approximately 55 kD after deglycosylation with *N*-glycosidase F). Thus, an LRP fragment generated by a presenilin-dependent cleavage can have a molecular weight that is, for example, less than about 85 kD, 80 kD, 75 kD, 70 kD, 65 kD, 60 kD, 55 kD, 50 kD, 45 kD, 40 kD, 35 kD, 30 kD, 25 kD, 20 kD, 15 kD or 10 kD or less. LRP fragments that are particularly indicative of a presenilin-dependent cleavage have a molecular weight that is less than about 15 kD, 13 kD, 12 kD, 10 kD or 5 kD.

In a particular embodiment of a method for assessing presenilin activity provided herein, LRP processing in a sample for which presenilin activity is being assessed can be evaluated by evaluating the LRP composition to determine if any fragment(s) indicative of altered presenilin-dependent cleavage of LRP are present and/or the level of any such fragment(s). Altered presenilin activity can be, for example, an increase, reduction or elimination of presenilin activity. In a particular embodiment of this method, the presence or absence and/or the level of an LRP fragment that is cleaved in the presence of a presenilin-dependent activity (*e.g.*, presenilin-dependent γ-secretase activity), and thus absent (or present at low levels) in the presence of the presenilin-dependent activity, but that can be detected intact when the presenilin-dependent activity is altered (such that it is reduced or eliminated) is assessed. One such fragment indicative of altered presenilin-dependent cleavage has a molecular weight of between about 25 kD and 15 kD, and, in particular, about 20 kD. The fragment can be one that is cleaved in the presence of a presenilin-dependent activity in such a way as to eliminate an amino acid sequence in a C-terminal region of LRP that is recognized by C-terminus-reactive antibody (i.e., the cleavage in the presence of a presenilin-dependent activity eliminates an epitope in the fragment that is recognized by an antibody generated against the C-terminal 13-amino acids of LRP). The LRP fragment can be one that contains an amino acid sequence located within the sequence of amino acids 4420-4544 of SEQ ID NO:10.

As described herein above, LRP is also cleaved by activities that are not presenilin dependent. Specifically, in one cleavage that is not a presenilin-dependent/γ-

secretase activity, mature LRP, i.e., separate, but noncovalently associated, α (N-terminal 515 kD extracellular subunit) and β (C-terminal 85 kD membrane-anchored subunit) chains, is generated by proteolytic cleavage at amino acid position 3925 (C-terminal to the tetrabasic amino acid sequence RHRR) of the 600-kD precursor polypeptide (see SEQ ID NO: 10 and GenBank Accession No. Q07954) in a process that involves the endoproteinase furin. Another cleavage of LRP that is not presenilin-dependent is the metalloproteinase-mediated proteolytic processing at the cell surface which results in "shedding" from the cell surface of a portion of LRP containing the α chain (an ~500-kD soluble polypeptide) that is noncovalently associated with a truncated β chain (the extracellular portion of the β chain (i.e., amino acids 3944-4420 of SEQ ID NO: 10; Mr =~67 kD or Mr =~55 kD after deglycosylation with *N*-glycosidase F). A fragment such as these that does not result from a presenilin-dependent cleavage generally is not alone indicative of presenilin activity.

In methods for assessing presenilin activity provided herein that include a step of determining the level of one or more fragments of LRP and/or LRP composition, LRP protein and/or fragments thereof can be detected and/or measured by any method known to those of skill in the art for measuring protein level or by any method described herein. In a particular embodiment of the method, LRP protein or a peptide fragment thereof is detected by immunoassay. For example, an LRP fragment from a C-terminal portion of LRP is visualized by immunoblotting of cell lysates with the anti-LRP polyclonal antibody (R9377) prepared to the carboxyl-terminal 13 amino acid peptide of LRP (C-GRGPEDEIGDPLA) as described in the EXAMPLES.

e. Methods for identifying or screening for agents that modulate presenilin activity

Methods for assessing presenilin activity provided herein can be applied to the identification of or screening for agents that modulate presenilin activity. One method provided herein for identifying or screening for agents that modulate presenilin activity includes steps of contacting a sample containing a presenilin and a lipoprotein receptor-related protein (LRP) and/or portion(s) or fragment(s) thereof with a test agent and identifying an agent that alters the processing and/or cleavage of an LRP or fragment

thereof.

A sample that can be used in the methods of identifying an agent that modulates presenilin activity can be any composition (*e.g.*, a biological or physiological composition) that includes a source of presenilin and a source of LRP and/or portion(s) thereof. Examples of samples include, but are not limited to, a cell, a cell extract or lysate, a cellular membrane and a cell-free medium.

(1) Presenilin and LRP (and/or portion(s) thereof)

Sources of presenilin and LRP include, but are not limited to: a cell that expresses endogenous or heterologous presenilin and/or LRP; a cell that expresses a recombinant portion(s) or fragment(s) of presenilin and/or LRP; lysates, extracts, or membrane fractions of any such cells; presenilin, LRP, or a portion thereof, that is isolated from such cells; and synthetic presenilin or LRP protein or synthetic proteins that represent a portion of presenilin or LRP.

Compositions, and methods of making compositions, that are sources of presenilin, LRP, and portion(s) thereof, are described herein and known in the art. For example, cells that endogenously express presenilin and/or LRP are known in the art as are nucleic acids encoding presenilin (see, *e.g.*, SEQ ID NOs: 5 and 7) and LRP (see, *e.g.*, SEQ ID NO: 9) that can be used to express the encoded proteins in cells. Methods of preparing lysates, extracts and membrane fractions of such cells are also described herein and known in the art, as are synthetic methods for generating proteins and peptides and preparatory methods of isolating proteins and peptides.

(2) Identifying an agent that alters the processing and/or cleavage of LRP (or portion(s) thereof)

In general, the step of identifying an agent that alters the processing and/or cleavage of LRP (or portion(s) thereof) can involve a comparison of the cleavage and/or processing of LRP (and/or portion(s) thereof) in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). If the cleavage and/or processing of LRP (and/or portion(s) thereof) in the test and control samples differs, then the agent is identified as one that modulates presenilin activity. For example, processing of LRP and/or the level of a

particular fragment of LRP in the test and control samples may differ by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or more than 75%. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

The processing or cleavage of an LRP or fragment(s) thereof can be assessed by determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP using, for example, materials and methods described herein. Thus, the LRP composition can be evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage of LRP or altered presenilin-dependent cleavage of LRP are present and/or the level of any such fragments. Such fragments and compositions are described herein. In a particular embodiment, the processing or cleavage of an LRP or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that is cleaved in the presence of a presenilin-dependent activity (presenilin-dependent γ-secretase activity), and thus absent (or present at low levels) in the presence of the presenilin-dependent activity, but that can be detected intact when the presenilin-dependent activity is altered (such that it is eliminated or reduced). In one embodiment, the presence or absence and/or level of an LRP fragment having a molecular weight of between about 25 kD and 15 kD, and, in particular, about 20 kD, is assessed. Typically, the ~ 20 kD fragment is one that is present when an LRP is not cleaved by a presenilin-dependent activity, such as one that occurs in the presence of an inhibitor of a presenilin-dependent activity such as DAPT. In a particular embodiment, the fragment is from a C-terminal portion of LRP, i.e., a CTF. The LRP fragment can be one that contains an amino acid sequence located within the sequence of amino acids 4420-4544 of SEQ ID NO:10. In a further embodiment, the fragment is one that is recognized by an antibody generated against C-terminal amino acids (*e.g.*, the C-terminal 13 amino acids) of LRP, such as, for example, the polyclonal antibody R9377 described herein.

The methods for identifying an agent that modulates presenilin activity as provided and described herein can be applied to the identification of candidate agents for

the treatment or prophylaxis of a disease associated with an altered presenilin. A particular embodiment of this method includes steps of contacting a sample containing a lipoprotein receptor-related protein (LRP), and/or fragment(s) thereof, and an altered presenilin, and/or fragment(s) thereof, that is associated with an altered processing of LRP with a test agent and identifying a candidate agent that restores LRP processing substantially to the processing that occurs in the presence of a presenilin, and/or fragment(s) thereof, that is not associated with an altered processing of LRP. The altered presenilin, and/or fragment(s) thereof, can be one that has an altered function or activity. Altered presenilins include, for example, a presenilin and/or fragment(s) thereof containing a mutation and/or encoded by a polymorphic nucleic acid that contains a mutation. Thus, the altered presenilin and/or fragment(s) thereof, can be one that is altered relative to a wild-type presenilin. Typically, a wild-type protein, such as, for example, a presenilin protein, can be one that is encoded by a predominant allele in a population or any allele that is not associated with disease or a pathogenic condition. A wild-type presenilin can be one that occurs in an organism that exhibits normal presenilin-dependent LRP processing patterns. The altered presenilin can be, for example, one that is encoded by a nucleic acid linked to Alzheimer's disease. For example, the nucleic acid may include any one or more of the at least 60 mutations in human PS1 and the at least two mutations in human PS2 that have been genetically linked to early onset familial Alzheimer's disease (FAD). Exemplary presenilins with altered activity include FAD-associated mutant forms of PS1 and PS2 that give rise to an increased accumulation of Aβ42 in AD patients and transfected cell lines and transgenic animals in which they are expressed. Included among such mutations are the PS2 FAD mutation N141I (Volga German FAD mutant) and the PS1 FAD mutation M146L.

Examples of diseases associated with an altered presenilin for which the methods provided herein can be used to identify candidate therapeutic or prophylactic agents include, but are not limited to, amyloidosis-associated diseases and neurodegenerative diseases. In a particular aspect, the disease is Alzheimer's Disease.

The sample used in the methods can be any sample, including samples described herein for the methods of identifying agents that modulate presenilin activity. For

example, a sample can contain cell(s), tissue, a cell or tissue lysate or extract, a body fluid, a cell membrane or composition containing cell membranes and a cell-free extract or other cell-free sample. In a particular embodiment, the sample includes a cell that contains the presenilin and LRP.

In general, the step of identifying a candidate agent that restores LRP processing to the processing that occurs in the presence of a presenilin that is not associated with an altered processing of LRP can involve a comparison of the cleavage and/or processing of LRP (and/or portion(s) thereof) in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). If the cleavage and/or processing of LRP (and/or portion(s) thereof) in the test and control samples differs, then the test agent is identified as a candidate agent for the treatment and/or prophylaxis of a disease associated with an altered presenilin. For example, processing of LRP and/or the level of a particular fragment of LRP in the test and control samples may differ by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or more than 75%. Additionally or alternatively, the cleavage and/or processing of LRP in the test sample can be compared to that in a positive control sample. An example of a positive control is a sample containing LRP (and/or portion(s) thereof) and a presenilin that is not associated with an altered processing of LRP (or an unaltered or wild-type presenilin). In comparing the test sample to the positive control, a test agent is identified as a candidate agent for the treatment and/or prophylaxis of a disease if the cleavage and/or processing of LRP (and/or portion(s) thereof) in the test and positive control samples is substantially similar. LRP cleavage and/or processing in the test and positive control samples could be substantially similar if the LRP processing and/or cleavage in the test sample is more similar to that in the positive control sample than that in the control sample that contains the altered presenilin and that was not contacted with the test agent.

The cleavage and/or processing of LRP (and/or portion(s) thereof) in a sample can be assessed, for example, using any of the methods and compositions provided and described herein. Assessing cleavage and/or processing of LRP can provide an assessment of presenilin activity. The processing and/or cleavage of an LRP can be

assessed by determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP. In a particular embodiment, the processing or cleavage of the LRP or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that has a molecular weight of between about 25 kD and 15 kD, and, in particular, about 20 kD. The fragment can be one that is contained within a transmembrane region of LRP and/or binds with an antibody generated against a C-terminal amino acid sequence of an LRP, such as, for example, a sequence of about the C-terminal 13 amino acids of an LRP. The LRP fragment can be one that contains an amino acid sequence located within the sequence of amino acids 4420-4544 of SEQ ID NO:10. The fragment can be one that is present when an LRP is not cleaved by a presenilin-dependent activity, for example, as may occur in the presence of an inhibitor of a presenilin-dependent activity such as, for example, DAPT. In a further embodiment, the fragment is one that is recognized by an antibody generated against C-terminal amino acids (*e.g.*, the C-terminal 13 amino acids) of LRP, such as, for example, the polyclonal antibody R9377 described herein.

2. Notch NICD Assay

Notch is a single transmembrane domain cell surface receptor that facilitates many cell fate decisions during development, including neurogenesis. Although its function in mature cells is unclear, its presence in adult mammalian brain has been demonstrated, although at significantly lower levels than in embryonic brain (Berezovska *et al.*, 1998, *J. Neuropathol Exp Neurol. 57(8)*:738-45). In addition, a potential role in adult brain including neurite extension has been suggested (Berezovska *et al.*, 1999, *Brain Res. Mol. Brain Res. 69(2)*:273-80). Notch, as well as APP, has been found to form stable complexes with PS1 in transfected mammalian cells (Xia, W. *et al.*, 1997, *Proc. Natl. Acad. Sci. 94*:8208-8213; Ray, W.J., *et al.*, 1999, *Proc. Natl., Acad. Sci. 96*:3263-3268).

Notch is synthesized as a 300 kDa precursor molecule, full-length notch (FLN), and undergoes at least three different proteolytic processing events during maturation and signal transduction. The amino acid sequence of the notch precursor polypeptide is provided in SEQ ID NO: 32, and DNA encoding the polypeptide is provided in SEQ ID

NO: 31. In the trans-Golgi network lumen, FLN is cleaved by the protease Furin at a site in the extracellular domain. This cleavage generates two fragments that remain associated during transport to the cell surface forming a heterodimeric receptor at the cell surface. Ligand binding to the receptor triggers an additional cleavage of the extracellular region of the C-terminal domain shortening the extracellular region to 12 amino acids. A third presenilin-dependent proteolytic cleavage event occurs within the transmembrane domain and releases the nuclear intracellular carboxyl domain (NICD). A presenilin-dependent cleavage of Notch has been shown between residues G1743 and V1744 (SEQ ID NO: 32 or the amino acid sequence provided as GenBank Accession No. AF308602). NICD translocates to the nucleus and activates transcription of target genes that influence crucial cell fate decisions during development and particularly haematopoiesis.

In a method for assessing presenilin activity provided herein, the level of one or more fragments of Notch and/or the composition of Notch is determined for a sample for which presenilin activity is being assessed. Examples of a sample for which presenilin activity is being assessed include, but are not limited to, a cell that expresses presenilin, a lysate or extract of a cell that expresses presenilin, or membranes prepared from a cell that expresses presenilin. The cell can endogenously express presenilin and/or express heterologous presenilin. Notch can be added to the sample or can be expressed endogenously and/or heterologously by the cell. In a particular embodiment, the method includes assessing presenilin activity of a cell by evaluating the level (which includes determining the presence or absence of) of a fragment from a C-terminal portion of Notch in a cell lysate.

To assess presenilin activity in these methods, the processing of Notch is evaluated. In evaluating Notch processing, the composition of Notch can be evaluated. The composition of Notch refers to the make-up of any Notch that is present anywhere in the analysis mixture, including the assay medium in which the analysis is being performed, an extracellular medium, or a cell membrane, lysate or extract. Thus, in one embodiment, the structure of any Notch present can be evaluated to, for example, determine whether Notch is intact or has been processed and appears as a fragment or

fragments of sizes smaller than the intact Notch molecule. In evaluating Notch processing, the levels, and/or the presence or absence, of one or more Notch fragments can be determined.

In particular, the Notch composition is evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage (or altered presenilin-dependent cleavage) of Notch are present and/or the level of any such fragment(s). A presenilin-dependent cleavage described herein occurs within the C-terminal portion of Notch. Thus, a presenilin-dependent cleavage of Notch can be one that occurs in the C-terminal portion of Notch at a position C-terminal to amino acid position 1743 of SEQ ID NO: 32 (or of the amino acid sequence provided as GenBank Accession No. AF308602). The presenilin-dependent cleavage of Notch can be one that occurs within the sequence of the last approximately 850, 815, 800, 750, 700, 750, 700, 650, 600, 650, 600, 550, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 25, or less amino acids of Notch. The presenilin-dependent cleavage can be one that occurs C-terminal to the extracellular portion of Notch (i.e., C-terminal to amino acid 1727 of SEQ ID NO: 32 or of the amino acid sequence provided as GenBank Accession No. AF308602). The presenilin-dependent cleavage of Notch can be one that occurs near or within the region of the Notch protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of Notch can be one that generates a soluble intracellular peptide containing the extreme C-terminus of Notch and a membrane-associated peptide containing amino acid sequence of the transmembrane region of Notch, particularly the more C-terminal region of the transmembrane segment of Notch. A Notch fragment generated by a presenilin-dependent cleavage can be one that can be detected by a reagent that binds to or recognizes an amino acid sequence from a C-terminal portion of Notch.

As described herein above, Notch is also cleaved by activities that are not presenilin dependent. Specifically, in one cleavage that is not a presenilin-dependent/γ-secretase activity, Notch is cleaved at the cell surface which results in "shedding" from the cell surface of a portion of the extracellular segment of Notch. A fragment such as

this that does not result from a presenilin-dependent cleavage generally is not alone indicative of presenilin activity.

In particular embodiments, Notch processing by PS1/γ-secretase can be assessed by determining the levels and/or presence or absence of the Notch ICD peptide and/or the Notch membrane-associated peptide that result from presenilin-dependent cleavage of Notch. In addition, the level, presence or absence of a Notch fragment that occurs in the absence of presenilin-dependent cleavage of Notch can be determined. Notch peptide levels can be measured by any method known to those of skill in the art for measuring protein level or by any method described herein. In a particular embodiment of the method, Notch peptide levels are measured by immunoassay. Anti-Notch peptide antibodies for use in such immunoassays can be obtained by the methods described herein or known to those of skill in the art. For example, Myc-tagged Notch derivatives may be used and detected with monoclonal anti-Myc antibodies (i.e., 9E10 from ATCC) (Schroeter *et al.*, (1998) *Nature 39*: 382-386; Song *et al.*, (1999) *Proc. Natl. Acad. Sci. 96*: 6959-6963) or V5 antibody epitope tagged Notch derivatives may be used and detected with anti-V5 antibody as described in the EXAMPLES.

3. E-cadherin assay

E-cadherin controls a wide array of cellular behaviors including cell-cell adhesion, differentiation and tissue development. Presenilin has been shown to form complexes with the cadherin/catenin adhesion system resulting in cleavage and release of the E-cadherin intracellular domain and disassembly of adherens junctions (Baki *et al.* 2001, *Proc. Natl. Acad. Sci. 98(5)*:2381-2386; Marambaud *et al.* 2002, *EMBO J. 21(8)*:1948-56). The amino acid sequence encoding a full-length human E-cadherin polypeptide is provided in SEQ ID NO: 34, and DNA encoding the polypeptide is provided in SEQ ID NO: 33. A presenilin-1-dependent γ-secretase cleavage stimulated by apoptosis or calcium influx occurs between human E-cadherin residues Leu731 and Arg732 at the membrane-cytoplasm interface. The PS1/γ-secretase system cleaves both the full-length E-cadherin and a transmembrane C-terminal fragment, derived from a metalloproteinase cleavage after the E-cadherin ectodomain residue Pro700, approximately seven residues upstream of the transmembrane domain (i.e., amino acids

708-731 of SEQ ID NO: 34 or of the amino acid sequence provided as GenBank Accession No. NP_004351). Metalloproteinase cleavage of the N-terminus of full-length E-cadherin produces a 38 kDA fragment (E-Cad/CTF1) that binds both β-catenin and PS1. Full-length E-cadherin and E-Cad/CTF1 are found only in the membrane and cytoskeletal (Triton X-100-insoluble) fraction. Cleavage by PS1/γ-secretase defines the N-terminal region of a 33 kDa fragment (E-Cad/CTF2 or E-Cad intracellular carboxyl domain (ICD)) that binds only β-catenin. A PS1/γ-secretase cleavage of E-cadherin has been shown between residues Leu731 and Arg732 (SEQ ID NO: 34 or the amino acid sequence provided as GenBank Accession No. NP_004351) at the interface of the membrane with the cytoplasm (Marambaud *et al.* 2002, *EMBO J. 21(8)*:1948-56). E-Cad ICD localizes in the membrane and in the soluble cytosol. Cleavage of E-cadherin by caspase-3 between residues 750 and 751 has also been reported (Steinhusen *et al.* (2001) *J. Biol. Chem.*, 276:4972-4980). The PS1/γ-secretase cleavage dissociates E-cadherins, β-catenin and α-catenin from the cytoskeleton, thus promoting disassembly of the E-cadherin-catenin adhesion complex. Furthermore, this cleavage releases the cytoplasmic E-cadherin intracellular carboxyl domain (ICD) to the cytosol and increases the levels of soluble β- and α catenins. Thus, the PS1/γ-secretase system stimulates disassembly of the E-cadherin-catenin complex and increases the cytosolic pool of β-catenin, a key regulator of the Wnt signaling pathway involved in cell proliferation.

In a method for assessing presenilin activity provided herein, the level of one or more fragments of E-cadherin and/or the composition of E-cadherin is determined for a sample for which presenilin activity is being assessed (examples of which are described herein). E-cadherin can be added to the sample or, if the sample is a cell sample, E-cadherin can be expressed endogenously and/or heterologously by the cell. In a particular embodiment, the method includes assessing presenilin activity of a cell by evaluating the level and/or presence or absence of a fragment from a C-terminal portion of E-cadherin in a cell lysate.

To assess presenilin activity in these methods, the processing of E-cadherin is evaluated. In evaluating E-cadherin processing, the composition of E-cadherin can be evaluated. The composition of E-cadherin refers to the make-up of any E-cadherin that is

present anywhere in the analysis mixture, including the assay medium in which the analysis is being performed, an extracellular medium, or a cell membrane, lysate or extract. Thus, in one embodiment, the structure of any E-cadherin present can be evaluated to, for example, determine whether E-cadherin is intact or has been processed and appears as a fragment or fragments of sizes smaller than the intact E-cadherin molecule. In evaluating E-cadherin processing, the levels and/or presence or absence of one or more E-cadherin fragments can be determined.

In particular, the E-cadherin composition is evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage (or altered presenilin-dependent cleavage) of E-cadherin are present and/or the level of any such fragment(s). A presenilin-dependent cleavage described herein occurs within the C-terminal portion of E-cadherin. Thus, a presenilin-dependent cleavage of E-cadherin can be one that occurs in the C-terminal portion of E-cadherin at a position C-terminal to amino acid position 731 of SEQ ID NO: 34 (or of the amino acid sequence provided as GenBank Accession No. NP_004351). The presenilin-dependent cleavage of E-cadherin can be one that occurs within the sequence of the last approximately 151, 150, 100, 50, 25, or less amino acids of E-cadherin. The presenilin-dependent cleavage can be one that occurs C-terminal to the extracellular portion of E-cadherin (i.e., C-terminal to amino acid 707 of SEQ ID NO: 34 or of the amino acid sequence provided as GenBank Accession No. NP_004351). The presenilin-dependent cleavage of E-cadherin can be one that occurs near or within the region of the E-cadherin protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of E-cadherin can be one that generates a soluble intracellular peptide containing the extreme C-terminus of E-cadherin and a membrane-associated peptide containing amino acid sequence of the transmembrane region of E-cadherin, particularly the more C-terminal region of the transmembrane segment of E-cadherin. Any E-cadherin fragments generated by such presenilin-dependent activities would have a molecular weight that is less than that of the E-Cad/CTF1 fragment produced by metalloproteinase cleavage of the N-terminus of full-length E-cadherin (E-Cad/CTF1 molecular weight is

approximately 38 kDa). Also, because caspase-3 can cleave a portion of the fragment produced by presenilin dependent cleavage, the molecular weight of such a fragment may be further reduced. Thus, an E-cadherin fragment generated by a presenilin-dependent cleavage can have a molecular weight that is, for example, less than about 40 kD, 35 kD, 30 kD, 25 kD, 20 kD, 15 kD or 10 kD or less. In a particular embodiment, an E-cadherin fragment generated by a presenilin-dependent cleavage has a molecular weight of less than about 35 kD or that is about 33 kD. An E-cadherin fragment generated by a presenilin-dependent cleavage can be one that can be detected by a reagent that binds to or recognizes an amino acid sequence from a C-terminal portion of E-cadherin.

As described herein above, E-cadherin is also cleaved by activities that are not presenilin dependent. Specifically, in one cleavage that is not a presenilin-dependent/γ-secretase activity, full-length E-cadherin is cleaved by a metalloproteinase at the cell surface which results in "shedding" from the cell surface of a portion of the extracellular segment of E-cadherin (i.e., amino acids N-terminal of amino acid 701 of SEQ ID NO: 34 or of the amino acid sequence provided as GenBank Accession No. NP_004351). A fragment such as this that does not result from a presenilin-dependent cleavage is not alone indicative of presenilin activity.

In particular embodiments E-cadherin processing by PS1/γ-secretase can be determined by measuring the levels of the E-cadherin ICD peptide and/or the E-cadherin CTF1 peptide. In addition, the level, presence or absence of a Notch fragment that occurs in the absence of presenilin-dependent cleavage of Notch can be determined. For example, inhibition of the PS1/γ-secretase processing of E-cadherin may result in the accumulation of the CTF1 peptide and/or a decrease in the level of the ICD peptide. E-cadherin peptide levels can be measured by any method known to those of skill in the art for measuring protein level or by any method described herein. For example, levels of E-cadherin peptides may be measured by immunoassay using anti-E-Cad/CTF1 or anti-E-Cad ICD antibodies. Antibodies for use in such immunoassays can be obtained by the methods described herein or known to those of skill in the art such as those described by Marambaud *et al.* (*EMBO J. (2002) 21(8)*:1948-56)..

4. **Erb-B4 assay**

Erb-B4 is a type I membrane receptor tyrosine kinase, which belongs to the epidermal growth receptor family and mediates response to multiple growth factors, including neuregulins. Erb-B4 has been implicated in many important biological and pathological processes, such as cardiovascular, mammary gland, and neuronal development, as well as malignancy and heart disease. The amino acid sequence of the ~180 kDa full-length Erb-B4 polypeptide is provided in SEQ ID NO: 36, and DNA encoding the polypeptide is provided in SEQ ID NO: 35. Constitutive ectodomain shedding of full-length Erb-B4 by a metalloprotease yields an ~80 kDa membrane-associated C-terminal fragment (B4-CTF) and a ~120 kDa ectodomain N-terminal fragment that is released into the extracellular medium. B4-CTF is further cleaved by a presenilin dependent γ-secretase releasing the soluble intracellular domain of Erb-B4 ICD which translocates to the nucleus and may participate in activation of gene transcription. The Erb-B4 ICD is believed to be ~80 kDa and contain a tyrosine kinase domain. Cleavage has been shown to occur at conserved residue Val673 on the C-terminal side of the transmembrane domain (residues 649-675 of amino acid SEQ ID NO: 36). This cleavage site is topologically similar to the γ-secretase cleavage site in Notch and cleavage of APP at conserved residue Val49.

In a method for assessing presenilin activity provided herein, the level of one or more fragments of Erb-B4 and/or the composition of Erb-B4 is determined for a sample for which presenilin activity is being assessed. Examples of a sample for which presenilin activity is being assessed are described herein. Erb-B4 can be added to the sample or can be expressed endogenously and/or heterologously by a cell in the sample. In a particular embodiment, the method includes assessing presenilin activity of a cell by evaluating the level and/or presence or absence of a fragment from a C-terminal portion of Erb-B4 in a cell lysate.

To assess presenilin activity in these methods, the processing of Erb-B4 is evaluated. In evaluating Erb-B4 processing, the composition of Erb-B4 can be evaluated. The composition of Erb-B4 refers to the make-up of any Erb-B4 that is present anywhere in the analysis mixture, including the assay medium in which the analysis is being performed, an extracellular medium, or a cell membrane, lysate or extract. Thus,

in one embodiment, the structure of any Erb-B4 present can be evaluated to, for example, determine whether Erb-B4 is intact or has been processed and appears as a fragment or fragments of sizes smaller than the intact Erb-B4 molecule. In evaluating Erb-B4 processing, the levels and/or presence or absence of one or more Erb-B4 fragments can be determined.

The Erb-B4 composition can be evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage (or altered presenilin-dependent cleavage) of Erb-B4 are present and/or the level of any such fragment(s). A presenilin-dependent cleavage described herein occurs within the C-terminal portion of Erb-B4. Thus, a presenilin-dependent cleavage of Erb-B4 can be one that occurs in the C-terminal portion of Erb-B4 at a position C-terminal to Val673 of SEQ ID NO: 36 (or of the amino acid sequence provided as GenBank Accession No. AAB59446). The presenilin-dependent cleavage of Erb-B4 can be one that occurs within the sequence of the last approximately 650, 600, 550, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 25, or less amino acids of Erb-B4. The presenilin-dependent cleavage can be one that occurs C-terminal to the extracellular portion of Erb-B4 (i.e., C-terminal to amino acid 648 of SEQ ID NO: 36 or of the amino acid sequence provided as GenBank Accession No. AAB59446). The presenilin-dependent cleavage of Erb-B4 can be one that occurs near or within the region of the Erb-B4 protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of Erb-B4 can be one that generates a soluble intracellular peptide containing the extreme C-terminus of Erb-B4 and a membrane-associated peptide containing amino acid sequence of the transmembrane region of Erb-B4, particularly the more C-terminal region of the transmembrane segment of Erb-B4. Any Erb-B4 fragments generated by such presenilin-dependent activities would have a molecular weight that is less than that of the ~180 kDa full-length Erb-B4 polypeptide minus the ~120 kDa ectodomain N-terminal fragment that is released into the extracellular medium upon metalloproteinase cleavage. Thus, an E-cadherin fragment generated by a presenilin-dependent cleavage can have a molecular weight that is, for example, less than about 100 kD, 90 kD, 80 kD, 70 kD, 60 kD, 50 kD, 40 kD, 30 kD, 20

kD, 15 kD or 10 kD or less. In a particular embodiment, an Erb-B4 fragment generated by a presenilin-dependent cleavage has a molecular weight of less than about 90 kD or that is about 80 kD. An Erb-B4 fragment generated by a presenilin-dependent cleavage can be one that can be detected by a reagent that binds to or recognizes an amino acid sequence from a C-terminal portion of Erb-b4.

As described herein above, Erb-B4 is also cleaved by activities that are not presenilin dependent. Specifically, in one cleavage that is not a presenilin-dependent/γ-secretase activity, full-length Erb-B4 is cleaved by a metalloproteinase at the cell surface which results in "shedding" from the cell surface of a portion of the extracellular segment of E-cadherin (i.e., amino acids N-terminal of amino acid 648 of SEQ ID NO: 36 or of the amino acid sequence provided as GenBank Accession No. AAB59446). A fragment such as this that does not result from a presenilin-dependent cleavage generally is not alone indicative of presenilin activity.

In particular embodiments, Erb-B4 processing by PS1/γ-secretase can be assessed by determining the levels and/or presence or absence of the Erb-B4 ICD peptide and/or the Erb-B4 membrane-associated peptide. In addition, the level, presence or absence of an Erb-B4 fragment that occurs in the absence of presenilin-dependent cleavage of Notch can be determined. Erb-B4 peptide levels can be measured by any method known to those of skill in the art for measuring protein level or by any method described herein. In a particular embodiment of the method, Erb-B4 peptide levels are measured by immunoassay. Anti-Erb-B4 peptide antibodies for use in such immunoassays can be obtained by the methods described herein or known to those of skill in the art. For example, polyclonal antibodies to the carboxyl terminus (residues 1291-1308) can be purchased (Santa Cruz Biotechnology, Inc.). Other antibodies to Erb-B4 peptides have also been described (see, e.g., Ni, *et al.*, (2001) *Science 294*:2179-2181).

D. Methods of Identifying or Screening for Agents that Modulate Aβ Levels

Methods, and compositions for use therein, are provided for identifying or screening for agents that modulate the levels of one or more Aβ peptides in a sample. The sample may be any sample, such as described herein, and may be reflective of, *e.g.*, cellular and/or extracellular Aβ levels. In a particular embodiment, the methods can be

used to identify agents that modulate the levels of Aβ42, including cellular and/or extracellular Aβ42. In another embodiment, the methods can be used to identify an agent that selectively modulates the level of one or more Aβ peptides, such as, for example, Aβ42, including cellular and/or extracellular peptides. For example, in one embodiment, the method includes a step of identifying an agent that selectively modulates the level of one or two Aβ peptides relative to one or more other Aβ peptides. In a particular embodiment, an agent that selectively modulates the levels of Aβ42 only or of Aβ42 and Aβ39 only, relative to other Aβ peptides (including, *e.g.*, Aβ40, Aβ38 and/or Aβ43), is identified.

In another embodiment, the methods can be used to identify agents that modulate Aβ peptide levels (and, in particular, Aβ42 levels) without substantially affecting (or with limited, minimal or inconsequential effect on) compositions, mechanisms, processes and/or activities that are not significantly involved in the generation, degradation and/or clearance of one or more Aβ peptides. In a particular embodiment, the method involves a step of identifying an agent that modulates the levels (including cellular and/or extracellular) of one or more Aβ peptides without substantially altering the cleavage of a presenilin substrate, or portion thereof, that is not APP. In a further embodiment, the presenilin substrate is LRP. Included among the agents that can be identified using the methods provided herein are agents that modulate Aβ levels, for example, by modulating compositions (*e.g.*, proteases and proteins, such as proteins on which protease activities depend, including presenilins), mechanisms and/or activities involved in Aβ peptide formation, degradation and/or clearance in cells and/or extracellular medium without substantially affecting (or with only limited, minimal or inconsequential effect on) compositions, mechanisms and/or activities that are not significantly involved in Aβ peptide formation and persistence.

Agents identified by the methods provided herein have a variety of uses. For example, such agents can be used in elucidating the particular elements and pathways involved in Aβ peptide formation, degradation and clearance in cells. Such agents may be used to assess proteolytic processing in cells and to characterize enzyme and protein interactions that facilitate and/or inhibit such processing. Proteolytic processing events

include, but are not limited to, those involved in the production and/or degradation of Aβ peptides. For example, agents identified by the methods may be used to identify and/or characterize regulatory molecules including, but not limited to, proteases that produce or degrade Aβ peptides and proteins involved in the activation or inhibition of such proteases. In addition, because release of Aβ peptides is a normal event in virtually every cell, the agents identified herein can be used to further characterize the role of such peptides in biochemical pathways and/or normal cellular processes. The agents identified by the methods provided herein may also serve as candidate agents for the treatment and/or prevention of disorders and diseases characterized by and/or involving inappropriate levels or misregulation of Aβ. Such diseases and disorders include any disease or disorder involving misregulation of Aβ production, clearance, and/or degradation. Exemplary disease and disorders include neurodegenerative diseases and disorders, such as, but not limited to, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, amylotrophic lateral sclerosis (ALS), Down's syndrome, Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch Type (HCHWA-D), and advanced aging of the brain. Such agents can provide therapeutic and/or preventative benefit with limited-to-no potential side effects that can result from non-specific modulation of Aβ peptide processing.

The methods provided herein for identifying or screening for agents that modulate Aβ levels can be used to identify agents that modulate cell and/or cellular membrane (i.e., referred to herein as cellular) Aβ levels and/or extracellular Aβ levels. In general, the methods include steps of contacting a sample containing amyloid precursor protein (APP), and/or portion(s) thereof (*e.g.*, one or more Aβ peptides), with a test agent and identifying an agent that alters the Aβ peptide-producing cleavage of the APP, the processing of the APP, the processing of Aβ and/or the levels of one or more Aβ peptides in the sample.

The step of identifying an agent that alters the Aβ peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of Aβ and/or the levels of one or more Aβ peptides in the sample can be carried out in a number of ways. In general, the identification step can involve a comparison of the cleavage or processing of

APP (and/or portion(s) thereof), processing of Aβ and/or the Aβ levels of a sample that has been contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the Aβ-producing cleavage or processing of APP (and/or portion(s) thereof), the processing of Aβ and/or the Aβ levels of the test and control samples differ, then the agent is identified as one that modulates the level of one or more Aβ peptides. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent. Assessing the cleavage and processing of APP (and/or portion(s) thereof), the processing of Aβ, and the Aβ levels of a sample can be conducted in a number of ways such as described herein or known in the art. In a particular method for assessing the Aβ42 level of a sample, a monoclonal antibody provided herein that selectively binds Aβ42 relative to other Aβ peptides is used to in an immunoassay for the detection and/or quantitation of Aβ42.

The methods provided herein for identifying or screening for agents that modulate Aβ levels can also include identifying an agent that alters the Aβ peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of Aβ and/or the levels of one or more Aβ peptides in the sample without substantially altering the cleavage of a presenilin substrate, or portion thereof, other than APP. In these methods, a sample containing a source of a presenilin substrate (or a portion thereof) other than APP is contacted with the test agent. The sample may be the same as the sample containing APP (and/or portion(s) thereof) or can be a different sample. The process of identifying an agent that alters the Aβ peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of Aβ and/or the levels of one or more Aβ peptides in a sample can be carried out in a number of ways as described herein. In addition, the process of further identifying an agent that also does not substantially alter the cleavage of a presenilin substrate (other than APP), or portion thereof, can be carried out in a number of ways. In general, this process can involve a comparison of the presenilin-dependent cleavage and/or processing of a presenilin substrate (or portion thereof) other than APP and/or the levels of a peptide fragment or fragments of the presenilin substrate that is other than APP of a sample that has been

contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the presenilin-dependent cleavage or processing of the presenilin substrate that is other than APP and/or the substrate fragment(s) levels of the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the cleavage of the presenilin substrate, or portion thereof, that is other than APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

1. Samples for use in methods of identifying Aβ-modulating agents

A sample that can be used in the methods of identifying an agent that modulates the Aβ levels can be any composition (*e.g.*, a biological or physiological composition) that includes a source of APP, and/or portion(s) thereof, or a source of one or more Aβ peptides including, but not limited to, a cell, a cell extract or lysate, a cellular membrane and a cell-free medium. When the sample contains a source of APP, it generally also contains a source of enzymatic and/or other activity that provides for processing of APP, and, in particular, Aβ peptide-producing cleavage activity. When a sample is one for use in methods that include a step of identifying an agent that alters the processing, such as degradation, of Aβ, and thus contains a source of Aβ peptides, it generally also contains a source of enzymatic and/or other activity that provides for processing of Aβ (*e.g.*, a catabolic activity that degrades Aβ).

a. APP or portion(s) thereof

The APP, and/or portion(s) thereof, provided by the source contained within the sample is generally any APP (and/or portion(s) thereof) that include(s) the Aβ peptide domains within its amino acid sequence. Aβ peptides include, but are not limited to, (1) a peptide that results from processing or cleavage of an APP and that is amyloidogenic, (2) one of the peptide constituents of β-amyloid plaques, (3) a fragment or portion of the 43-amino acid sequence set forth in SEQ ID NO: 4 and (4) a fragment or portion of a peptide as set forth in (1) or (2). Aβ peptides derived from proteolysis of APP, or degradation of Aβ, generally are typically 39 to 43 amino acids in length (see, *e.g.*, SEQ

ID NO: 4 showing the 43-amino acid sequence of an Aβ peptide), depending on the carboxy-terminal end-point, which exhibits heterogeneity. However, Aβ peptides containing less than 39 amino acids, *e.g.*, Aβ39, Aβ38, Aβ37 and Aβ34, also may occur. Aβ peptides include those that begin at position 672 of APP770 (see SEQ ID NO: 2). Isoforms of APP that contain an Aβ domain include APP770, APP751, APP714, APP695, L-APP752, L-APP733, L-APP696 and L-APP697. APP can be an APP of any species. In particular embodiments, the APP is a mammalian APP, such as, for example, a rodent or human APP.

In methods of identifying or screening for agents that modulate Aβ levels that include a step of identifying an agent that alters the Aβ peptide-producing cleavage of APP, the sample can contain a source of APP that can be cleaved or modified to yield one or more Aβ peptides. In methods that include a step of identifying an agent that alters the processing, such as degradation, of Aβ, the sample generally contains a source of Aβ peptides. Such a source can be, for example, synthetic, recombinant or isolated Aβ peptides, or a source of APP that can be cleaved or modified to yield one or more Aβ peptides. In methods that include a step of identifying an agent that alters the processing of APP, the sample can contain a source of APP that can undergo processing. In methods that include a step of identifying an agent that alters the level of one or more Aβ peptides, the sample generally contains a source of Aβ peptides. Such a source can be, for example, synthetic, recombinant or isolated Aβ peptides, or a source of APP that can be cleaved or modified to yield one or more Aβ peptides.

Sources of APP, or a portion thereof, include, but are not limited to: a cell that expresses endogenous or heterologous APP; a cell that expresses a recombinant portion(s) or fragment(s) of APP; lysates, extracts, or membrane fractions of any such cells; APP, or a portion thereof, that is isolated from such cells; and synthetic APP protein or synthetic proteins that represent a portion of APP.

Sources of Aβ peptides include, but are not limited to: a cell that expresses endogenous or heterologous APP and enzymatic activities that cleave APP to yield Aβ peptides (*e.g.*, β- and γ-secretases); a cell that expresses recombinant Aβ peptides; lysates, extracts, or membrane fractions of any such cells; Aβ peptides that are isolated

from such cells; synthetic or isolated APP that is degraded to yield Aβ peptides; and synthetic Aβ peptides.

Compositions, and methods of making compositions, that are sources of APP, portion(s) thereof, and Aβ peptides are described herein and known in the art. For example, cells that endogenously express APP and/or Aβ peptides are known in the art as are nucleic acids encoding APP (or portion(s) thereof) and/or Aβ peptides (see, *e.g.*, SEQ ID NOs: 1, 3, 27 and 29) that can be used to express the encoded proteins in cells. Methods of preparing lysates, extracts and membrane fractions of such cells are also described herein and known in the art, as are synthetic methods for generating proteins and peptides and preparatory methods of isolating proteins and peptides.

b. Sources of activities that provide for processing of APP and/or Aβ peptides

Sources of activities that provide for cleavage or processing of APP (or portion(s) thereof) and/or Aβ peptides include, but are not limited to: a cell that expresses endogenous or heterologous molecules that give rise to the activities; lysates, extracts, or membrane fractions of any such cells; molecules that give rise to the activities that are isolated from such cells; and synthetic molecules that give rise to the activities.

Molecules that can be involved in activities that provide for cleavage or processing of APP or Aβ include, but are not limited to, secretases, including α-, β- and γ-secretase, presenilins, including PS1 and PS2, insulin-degrading enzyme (IDE), neprilysin, plasmin, uPA/tPA, endothelin converting enzyme-1, and matrix metalloproteinase-9 (see, *e.g.*, Selkoe (2001) *Neuron 32*:177-180; Vekrellis *et al.* (2000) *J. Neurosci. 20*:1657-1665; Iwata *et al.* (2000) *Nat. Med. 6*:143-150; Carson and Turner (2002) *J. Neurochem 81(1)*: 1-8; and Eckman *et al.* (2001) *J. Biol. Chem. 276*: 24540-24548). Such molecules can be from any species. In particular embodiments, the molecule is a mammalian molecule, such as, for example, a rodent or human molecule.

c. Conditions that enhance Aβ production

When a sample is one for use in methods that include a step of identifying an agent that alters the processing of Aβ and/or the levels of one or more Aβ peptides, it is generally desirable for the sample to contain a readily detectable amount of Aβ peptide.

To enhance Aβ production in a sample containing an Aβ-producing source, one or more modulatory molecules or compounds that provide for increased Aβ levels through increased Aβ production or decreased Aβ clearance can be included in the sample. For example, a modulatory molecule may function to activate β-secretase and/or γ-secretase contained within the sample for increased processing of APP into Aβ peptides. Alternatively, a modulatory molecule may function to inhibit one or more Aβ-degrading proteases leading to decreased clearance of Aβ peptides. Exemplary modulatory molecules of this kind may include, but are not limited to serine protease inhibitors such as α1-antichymotrypsin (Mucke *et al.* (2000) *An. J. Pathol. 157*: 2003-2010; Nilsson *et al.* (2001) *J. Neurosci. 21*:1444-1451). In addition, the protease inhibitor thiorphan which is known to inhibit several proteases, has been shown to induce plaque formation in rats (Iwata *et al.* (2000) *Nat. Med. 6*: 143-150).

d. Medium

A sample medium can be any medium in which APP, portion(s) thereof, and/or Aβ peptides can exist. Examples of sample medium include, but are not limited to, cells, cell lysates, extracts and membranes, and cell-free medium.

(1) Cells

(a) General features of cells

Although any cell may be used in the methods, cells that are particularly suitable are those that exhibit APP and/or Aβ peptide synthesis and processing and/or those in which Aβ levels and/or processing may readily be assessed. If a cell has an APP processing and/or cleavage activity but does not express APP (or expresses APP at only low or undetectable levels), nucleic acid encoding APP can be introduced into the cells, and vice versa. If a cell has an Aβ catabolic activity (i.e., an activity that degrades one or more forms of Aβ) but does not express Aβ (or expresses only low levels of Aβ or only particular forms of Aβ), nucleic acid encoding one or more Aβ peptides can be introduced into the cells, and vice versa. Cells that express enzymatic and/or other activities involved in APP and/or Aβ processing can also be used in conjunction with another or separate source of APP and/or Aβ peptides in the sample. Thus, transfected or

recombinant cells, as well as cells that endogenously express desired proteins and/or activities, can be used in the methods of identifying agents that modulate Aβ levels.

In particular examples, cells used in samples for the methods of identifying agents that modulate Aβ levels are eukaryotic cells. In a further example, the cells can be mammalian cells. Mammalian cells include, but are not limited to, rodent (*e.g.*, mouse, rat and hamster), primate, monkey, dog, bovine, rabbit and human cells. In particular embodiments of the methods, the sample includes a mammalian cell, such as, for example, a rodent or human cell, that expresses endogenous and/or heterologous APP (or a portion(s) thereof) and/or Aβ, and the activity or activities for processing and cleavage of APP and/or Aβ. Cells may also be cells of *in vivo* or *in vivo*-derived samples, including body fluids, such as but not limited to, serum, blood, saliva, cerebral spinal fluid, synovial fluid and interstitial fluids, urine, sweat and other such fluids and secretions.

Another feature of cells that are particularly suitable for use in the screening and identification methods is amenability to transfection/transformation with heterologous nucleic acid and amenability to gene expression alteration. A number of techniques for the introduction of heterologous nucleic acid into cells and for altering gene expression in cells are known in the art and described herein. The relative ease with which these techniques may be applied to a cell to effect recombinant expression of a heterologous nucleic acid, or reduction, alteration or elimination of one or more genes in the cell is a consideration in selection of cells for use in the methods provided herein. Amenability to gene expression alteration and analysis of Aβ may be considerations, for example, when screening agents in AD model systems (as described herein).

(b) Cells that exhibit APP and/or Aβ production

Exemplary cells that exhibit APP and/or Aβ production include, but are not limited to, primary cell cultures, typically neuronal cell cultures. Primary cells from any organism that exhibits APP and/or Aβ production and/or processing may be used. Examples include mixed fetal guinea pig brain cells (Beck (2000) *Neuroscience 95*:243-254). Primary cell cultures are harvested from a mammal and cultured using standard techniques and include cortical neural cells, microglia, glia, astrocytes, and the like.

Briefly, neural tissue including but not limited to the brain of a mammal expressing or diagnosed with AD symptoms is harvested, and optionally subjected to enzymatic digestion to ease the separation of cells. The cells can be mechanically separated as well. Cells can also be enriched by type or characteristic using standard techniques. Primary culture cells, typically neural tissue, can be induced to express Aβ in response to growth factors, cytokines, hormones, or transcription pathway activators. Thus, suitable cells include cells capable of expressing Aβ in response to an Aβ-inducing agent. An Aβ-inducing agent means any substance that causes and/or enhances the expression of APP or Aβ and includes, but is not limited, to growth factors including but not limited to TGF, TGF-β, PDGF, and EGF; cytokines, hormones or a combination thereof.

Totipotent, pluripotent, or other cells that are not terminally differentiated can be induced to express neuronal characteristics including the production of Aβ peptides. Exemplary non-terminally differentiated cells include embryonic stem cells, adult stem cells, mesenchymal stem cells, bone marrow stem cells, adipose tissue stem cells, and neuronal stem cells. These non-terminally differentiated cells can be induced to express Aβ when exposed to growth factors, cytokines, morphogenetic factors, or tissue specific inducing media. Thus, cells that can be used in the methods of identifying or screening for agents that modulate Aβ levels include non-terminally differentiated cells induced to express Aβ. The non-terminally differentiated cells can be of any lineage, endoderm, mesoderm, or ectoderm or a combination thereof.

Other cells that express APP and/or Aβ include immortalized cell lines transfected or transformed with exogenous nucleic acids encoding APP, Aβ, a precursor, or fragment thereof. For example, US Patent. No. 5,538,845, incorporated by reference, describes the transfection of chinese hamster ovary (CHO) cells and 293 human embryonic kidney (HEK) cell line, ATCC accession number CRL-1573, with cDNA encoding the 695, 751, and 770 amino acid isoforms of APP. Mouse neuroblastoma cells (*e.g.*, N2A cells; ATCC accession number CCL-131) are another example of cells that can be transfected with nucleic acid encoding APP, a portion(s) thereof or Aβ. Any of these cells can be cotransfected, if necessary, with vectors comprising nucleic acid sequences encoding β-secretase, γ-secretase and/or presenilin for the proeessing of APP

to generate Aβ peptides.

Additionally, SH-SY5Y cells, a human neuroblastoma cell line that secretes Aβ into the culture medium without βAPP transfection, can be used. This cell line is available from ECACC European Collection of Cell Cultures, CAMR Centre for Applied Microbiology & Research Porton Down, Salisbury, Wiltshire (UK) SP4 0JG UK under accession number 94030304.

Cells transfected with nucleic acid constructs can express APP and/or Aβ peptides using standard expression vectors. Expression can be, for example, constitutive or induced.

(2) Cell lysates, extracts and membranes and cell-free medium

Biological compositions that can be used as samples in the methods of identifying or screening for agents that modulate Aβ levels include, but are not limited to, purified or partially purified enzyme preparations, conditioned medium from cultured cells, cellular extracts and cell lysates. Cell lysates can be generated using methods described herein (see, *e.g.*, Example 8) and/or known in the art. For example, cell lysates can be prepared from cells able to process APP into Aβ and/or able to catabolize Aβ. Alternatively, appropriate APP processing or catabolic enzymes may be incubated with cell lysates devoid of such activity.

(3) *In vivo* systems

In addition, as described below, *in vivo* organism systems can also be used in methods of identifying Aβ-modulating agents. The organism can be one that produces endogenous APP and/or Aβ peptides and processing and cleavage activities or a transgenic organism (non-human) that has been generated to express heterologous APP and/or Aβ peptides and/or processing and cleavage activities. Organisms include, but are not limited to, mammals (*e.g.*, rodents) salmon (Maldonado *et al.* (2000) *Brain Res.* *858*:237-251), and invertebrate animals, for example, *Drosophila* and *C. elegans* (see, *e.g.*, Link (2001) *Mech. Ageing Dev.* *122*:1639-1649).

For example, in methods of identifying agents that modulate Aβ levels, an organism can be contacted with a test agent and the levels of Aβ in any sample from the

organism, *e.g.*, tissue, plasma, CSF and brain, can be compared between treated and untreated organisms. Plasma and CSF can be obtained from an organism using standard methods. For example, plasma can be obtained from blood by centrifugation, CSF can be isolated using standard methods, and brain tissue can be obtained from sacrificed organisms. The organism can be contacted with a test agent in various ways. For example, the test agent can be dissolved in a suitable vehicle and administered orally or by injection. The test agent also can be administered as a component of drinking water or feed.

2. Identification of agents that modulate Aβ levels

Cellular and extracellular Aβ levels, and the degree of Aβ accumulation, are dependent on Aβ production, through APP cleavage and processing, as well as on Aβ catabolism, degradation and clearance. A method for identifying or screening for agents that modulate Aβ levels can include steps of contacting a sample containing APP (and/or portion(s) thereof) with a test agent and identifying an agent that alters any one or more aspects of Aβ production and/or Aβ catabolism. Thus, the method can include a step of identifying an agent that alters the Aβ peptide-producing cleavage of APP, the processing of APP, the processing of Aβ and/or the levels of one or more Aβ peptides in a sample.

a. Assessment of Aβ peptide-producing cleavage of APP and APP processing

Any of the methods for identifying or screening for agents that modulate Aβ levels can include a step of assessing Aβ peptide-producing cleavage of APP and APP processing of a sample. For samples that contain a source of APP and of an APP-processing activity, a variety of methods are provided for assessment of Aβ peptide-producing cleavage of APP and APP processing. In a particular embodiment measurement of Aβ levels of the sample (as described in detail below) can provide a method for assessing Aβ peptide-producing cleavage of APP and APP processing. In other embodiments, measurement of APP fragments levels in a sample other than Aβ can be used as a means for assessing Aβ peptide-producing cleavage of APP and APP processing. In other embodiments, measurement of the activity of one or more enzymes in the sample can be used to assess Aβ peptide-producing cleavage of APP and APP

processing. The one or more enzymes are enzymes that participate in either the amyloidogenic or non-amyloidogenic APP cleavage pathways.

As described herein, APP can undergo proteolytic processing via two pathways: an amyloidogenic pathway and a non-amyloidogenic pathway. In the non-amyloidogenic pathway, cleavage of APP by α-secretase occurs at position 16 within the Aβ domain releasing the large N-terminal secreted ectodomain of APP ending at the α-secretase cleavage site (sAPPα) and a non-amyloidogenic C-terminal fragment of about 10 kD (C83; the 83-amino acid carboxyl tail of APP). Because α-secretase cleaves within the Aβ domain, this cleavage precludes Aβ formation. Rather, the C-terminal fragment of APP generated by α-secretase cleavage is subsequently cleaved by γ-secretase within the predicted transmembrane domain to generate a 22-24 residue non-amyloidogenic peptide fragment termed p3.

Alternatively, in the amyloidogenic pathway, cleavage of APP by β-secretase (BACE) occurs at the beginning of the Aβ domain defining the amino terminus of the Aβ peptide. This cleavage generates a shorter soluble N-terminus, APPβ, as well as an amyloidogenic C-terminal fragment (C99), the 99-amino acid C-terminal fragment that contains the transmembrane and cytoplasmic domains of APP. Further cleavage of this C-terminal fragment by γ-secretase, a presenilin-dependent enzyme, generates Aβ.

The activity of β-secretase versus α-secretase and, thus, the proportion of APP processed by these enzymes will affect the amount of Aβ produced. Swedish APP mutations have been mapped to the β-secretase cleavage site in APP and favor β secretase cleavage of APP. Thus, cells expressing these mutations secrete increased amounts of Aβ and decreased amounts of p3 as compared with cells expressing wild-type APP. In contrast to the Swedish mutation, which increases β-secretase cleavage, activation of protein kinase C (PKC) by phorbol 12-myristate 13-acetate (PMA) has been shown to favor α-secretase cleavage at the expense of β-secretase cleavage (Skovronsky *et al.*, (2000) *J. Bio. Chem. 275*: 2568-2575) indicating that PKC-regulated α-secretase competes directly with β-secretase for cleavage of APP. Furthermore, changes in levels of APP-CTFs have been shown to mirror changes seen in sAPPβ and sAPPα (e.g.,

increased levels of Aβ or decreased levels of p3 can be indicated by an increase in sAPPβ or by a similar decrease in sAPPα)

Since β-secretase activity may be limited by the availability of APP, then increased cleavage of APP by other secretases could decrease β-secretase cleavage of APP and hence Aβ production. Also, by the same reasoning, decreased cleavage of APP by other secretases could increase β-secretase cleavage of APP leading to increased Aβ production. It can, therefore, generally be assumed that an alteration in the non-amyloidogenic pathway will result in a similar but opposite alteration in the amyloidogenic pathway. Thus, agents that modulate enzymes or the regulation of enzymes in either the amyloidogenic or non-amyloidogenic pathway can modulate levels of Aβ. As a result, peptide-producing cleavage of APP and APP processing may be assessed by measuring the activity of such enzymes. Assessment of the activity of such enzymes can provide information about peptide-producing cleavage of APP and APP fragment production pattern (i.e., the types and amounts of APP peptide fragments produced by APP fragment production enzymes). Alternatively, assessment of peptide fragments (particularly non-Aβ peptide fragments) produced in both pathways (APP fragment production patterns) can provide information about the activities of enzymes in the pathways and peptide producing cleavage of APP. In a particular embodiment, Aβ peptide-producing cleavage of APP can be assessed by monitoring the activity of enzymes and/or the cleavage of APP by enzymes of the non-amyloidogenic pathway, specifically α-secretase activity and/or the levels of fragments generated by α-secretase activity including sAPPα, C83 and p3 peptide fragments. Likewise, agents that alter the Aβ peptide-producing cleavage of APP and APP processing may be screened for by monitoring enzyme activities and/or fragmentation patterns in the presence and absence of test agents.

b. Assessment of Aβ processing

Any of the methods for identifying or screening for agents that modulate Aβ levels can include a step of assessing Aβ processing of a sample. For samples that contain a source of APP and an APP-processing activity, methods such as those described above can provided for assessment of Aβ processing. For samples that contain

a source of Aβ and of an Aβ degradation activity, a variety of methods are provided for assessment of Aβ processing. In a particular embodiment measurement of Aβ levels of the sample (as described in detail below) can provide a method for assessing Aβ processing. In other embodiments, measurement of the activity of one or more degradation and/or clearance pathways and/or degradation fragment patterns in the sample can be used to assess Aβ processing. The one or more pathways include, but are not limited to, proteolytic degradation, receptor-mediated clearance, non-receptor-mediated clearance, and/or aggregation/fibrillogenesis. Defects in pathways for Aβ degradation and clearance can lead to an alteration in the levels of Aβ and, therefore, could underlie some or many cases of amyloidosis and other neurodegenerative disease such familial and sporadic AD as well as other diseases and disorders characterized by misregulation of Aβ. Aβ processing may, therefore, be assessed by monitoring enzyme activities involved in the degradation and clearance of Aβ. In addition, fragmentation patterns of Aβ produced upon cleavage by degradative enzymes may be used to assess Aβ processing. There are numerous proteases in the brain that could potentially participate in Aβ turnover, and there is evidence that several enzymes may contribute to the degradation of Aβ peptides in brain tissue including insulin-degrading enzyme (IDE), neprilysin, plasmin, uPA/tPA, endothelin converting enzyme-1, and matrix metalloproteinase-9 (Selkoe J. (2001) *Neuron 32*:177-180). Similarly, agents that alter Aβ processing may be screened for by monitoring the activity of one or more enzymes involved in the degradation and/or clearance of Aβ and/or fragmentation patterns of resulting degradation products in the presence and absence of test agents.

c. Assessment of Aβ levels

Any of the methods for identifying or screening for agents that modulate Aβ levels can include a step of assessing Aβ levels of a sample. For samples that contain a source of APP and of an APP-processing activity, an assessment of Aβ levels of the sample can provide a method for assessing Aβ peptide-producing cleavage of APP and for assessing APP processing. For samples that contain a source of Aβ peptides and of Aβ catabolic activity, an assessment of Aβ levels of the sample can provide a method for assessing processing of Aβ. For samples that contain a source of APP, Aβ, APP-

processing activity, and a source of Aβ catabolic activity, an assessment of Aβ levels of the sample can provide a method for assessing the overall balance of Aβ peptide-producing cleavage of APP, APP processing and Aβ processing.

In assessing the Aβ levels of a sample, the total Aβ (i.e., all forms of Aβ) level can be assessed in an indiscriminant determination of the Aβ level of a sample, or the level of one or more specific forms of Aβ can be assessed. In one embodiment of the methods, the level of Aβ42, Aβ40, Aβ39 and/or Aβ38 is assessed. In a particular embodiment, the level of Aβ42 is assessed.

Methods and compositions for indiscriminant assessment of total Aβ levels and for selective assessment of a particular Aβ peptide are provided herein. In a method provided herein for indiscriminant assessment of total Aβ levels, the sample, or portion thereof, is contacted with an antibody that binds to forms of Aβ that contain amino acids 1-12 of SEQ ID NO: 4. Also provided is an antibody that binds to forms of Aβ that contain amino acids 1-12 of SEQ ID NO: 4. In a method provided herein for the selective assessment of Aβ42 levels, the sample, or portion thereof, is contacted with an antibody that selectively binds to Aβ42 (*e.g.*, the sequence of amino acids 1-42 of SEQ ID NO: 4) relative to other forms of Aβ. Also provided is an antibody, and portions thereof, that selectively bind to Aβ42 relative to other forms of Aβ.

The Aβ levels of a sample or any portion(s) thereof may be assessed in the methods. For example, if the sample is a cell-free medium or culture medium, the Aβ levels of the medium can be assessed. If the sample is a cell sample, the Aβ levels of the extracellular medium (*e.g.*, secreted Aβ) of the sample and/or the cellular (*e.g.*, intracellular and/or membrane-associated Aβ) Aβ levels can be assessed. To assess the cellular Aβ levels, lysates, extracts, and/or membranes of the cells can be analyzed for Aβ protein. If the sample is an organism, then the cellular, tissue, and/or secreted Aβ levels can be assessed. For example, secreted Aβ levels could be assessed in fluids of the organism, such as, for example, any bodily fluids. Levels of secreted Aβ may be monitored, for example, by the methods described in Example 6. Preparation of whole cell lysates and membrane fractions are well known to those of skill in the art. Cell lysates may be obtained for instance by the method described in Example 8 for the

identification of LRP-CTFs.

(1) **Procedures for assessing Aβ levels**

Assessment of the Aβ level of a sample or portion(s) thereof can be conducted using methods described herein or any method known in the art for detecting the presence of and/or measuring the level or amount of a peptide or protein in a sample. For example, immunological detection techniques employing binding substances such as antibodies, antibody fragments, recombinant antibodies, and the like, can be used. Detection of Aβ peptide can be carried out using any standard antibody-based assays. Exemplary immunoassays are described in detail, for example, in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include, for example, concurrent immunoelectrophoresis, radioimmunoassay, immunoprecipitation, western hybridization, and enzyme-linked immunosorbent assays (ELISA), inhibition or competition assay, and sandwich assay. Suitable immunological methods employing a single antibody are also contemplated, for example, radioimmunoassay using an antibody specific for a particular form of Aβ, or single antibody ELISA methods.

Mass spectrometry and electrophoretic analysis of at least partially purified Aβ peptides are also techniques that can be used to detect and quantitate Aβ. In addition, the levels of different forms of Aβ can be quantified using known methods such as, for example, using internal standards and/or calibration curves generated by performing the assay with known amounts of standards.

(2) **Immunological methods for Aβ detection**

Aβ peptides, which can differ by only a single amino acid, can be fairly similar in molecular weight. Therefore, methods, such as immunological methods, that are based in detecting properties of Aβ peptides that can be more distinctive than molecular weight (at least when using standard and relatively inexpensive laboratory reagents and equipment) can be well-suited for assessing the level of a particular Aβ peptide. Methods and compositions for use in immunoassays for Aβ peptides in general are described herein.

Compositions and methods for detecting Aβ peptides that contain the sequence of

amino acids 1-12 of SEQ ID NO: 4, or a portion of this sequence, are provided herein. The compositions and methods are based on the generation of antibodies against a peptide having the amino acid sequence of amino acids 1-12 of SEQ ID NO: 4. In a particular embodiment, the antibody is B436, or a fragment thereof (see Examples 2 and 4). Because most Aβ peptides contain such a sequence, these compositions and methods are particularly useful in assessing the total Aβ content of a sample and in detecting most forms of Aβ.

More particularly, compositions and methods for detecting Aβ42 or assessing the Aβ42 content of a sample are provided herein. The compositions and methods are based on the development of an antibody that selectively binds Aβ42 relative to other Aβ peptides. In a particular embodiment, the antibody is A387, or a fragment thereof (see Examples 1 and 4).

(a) Antibody preparation

Antibodies specific for Aβ may be prepared against a suitable antigen or hapten comprising the desired target epitope. The target epitope may include any number of amino acids within any portion of an Aβ amino acid sequence. SEQ ID NO: 4 provides the amino acid sequence of a 43-amino acid form of a human Aβ (Aβ43). Shorter forms of human Aβ peptides include, but are not limited to, those having the amino acid sequence of amino acids 1-42, 1-40, 1-39, 1-38, 1-37 and 1-34 of SEQ ID NO: 4. Typically, the target epitope will include at least 2 contiguous residues and may include more than 6 contiguous residues within any portion of the Aβ amino acid sequence. The target epitope may include a sequence of amino acids from the amino terminus typically any of amino acids 1-13, the junction region typically containing any of the amino acids residues 13-26 and the carboxy terminus typically containing any of the amino acid residues 33-42.

A target epitope composed of such peptide fragments may be prepared, for example, from mammals such as humans, monkeys, rats and mice by methods which are known to those of skill in the art, and may also be purified natural samples which are commercially available. Partial peptides can be obtained by hydrolyzing longer forms of Aβ successively from the N-terminus and/or the C-terminus with exoproteases such as

aminopeptidase and carboxypeptidase or mixtures thereof or various endopeptidases or mixtures thereof.

Synthetic peptides may be prepared by methods known in the art including solid phase synthesis methods and liquid phase synthesis methods. Examples of such synthesis methods include methods described in Merrifield, (1963) *J. Am. Chem. Soc.* *85*:2149-2156; Bodanszky and Ondetti, *Peptide Synthesis*, Interscience Publishers, New York (1966); and Schroder and Lubke, *The Peptide*, Academic Press, New York, (1965). For example, when the Aβ peptides are synthesized by solid methods, any resins known in the art as insoluble resins (such as chloromethyl resins and 4-oxymethylphenylacetamidomethyl resins) are used for a successive condensation of protected amino acids to the C-terminal sides of the Aβ synthetic peptides according to usual methods. The protective groups are removed by hydrogen fluoride treatment, followed by purification by methods which are known in the art, such as high performance liquid chromatography. Thus, the desired Aβ peptides can be obtained.

N-protected amino acids can be produced by the methods of protecting the a-amino-groups with Boc groups; further, for example, the hydroxyl groups of serine and threonine with Bzl groups; the ω-carboxylic acid groups of glutamic acid and aspartic acid with OBzl groups; the ϵ-amino group of lysine with a Cl-Z group; the guanido group of arginine with a Tos group; and the imidazole group of histidine with a Bom group.

Once a sufficient quantity of peptide hapten has been obtained, it may be conjugated to a suitable immunogenic carrier. Natural polymer carriers can be used as immunogenic carriers and include, for example, albumin, thyroglobulin, hemoglobin, keyhole limpet hemocyanin, or other suitable protein carriers, as generally described in Hudson and Hay, *Practical Immunology*, Blackwell Scientific Publications, Oxford, Chapter 1.3, 1980. Examples of synthetic polymer carriers that can be used include various latexes of polymers or copolymers such as amino acid polymers, styrene polymers, acrylic polymers, vinyl polymers and propylene polymers. An exemplary immunogenic carrier utilized in the Examples provided herein is ovalbumin. Since Aβ peptides aggregate easily, insolubilized Aβ haptens can also be directly immunized without the use of a carrier.

In addition, various condensing agents can be used for coupling of the haptens and the carriers. Examples of the condensation agents include diazonium compounds such as bis-diazotized benzidine which crosslinks tyrosine, histidine and tryptophan; dialdehyde compounds such as glutaraldehyde which crosslinks amino groups together; diisocyanate compounds such as toluene-2,4-diisocyanate; dimaleimide compounds such as N,N'-o-phenylenedimaleimide which crosslinks thiol groups together; maleimide active ester compounds which crosslink amino groups and thiol groups; and carbodiimide compounds crosslinking amino groups and carboxyl groups. When amino groups are crosslinked together, there is another way in which an active ester reagent (for example, SPDP) having a dithiopyridyl group is reacted with one amino acid, followed by reduction to introduce a thiol group, whereas a maleimide group is introduced into the other amino group by the use of a maleimide active ester reagent, and then, both can be reacted with each other. Examples of such methods for crosslinking haptens with immunogenic carriers can be found, for example, in U.S. Patent Nos. 4,140,662 and 4,486,344.

Haptens can be used alone or together with carriers and diluents to produce antibodies specific for the desired epitope by in vitro or in vivo techniques. In vitro techniques involve exposure of lymphocytes to the immunogens, while in vivo techniques require the injection of the immunogens into a suitable vertebrate host. Suitable vertebrate hosts are non-human, including, for example, monkeys, dogs, guinea pigs, mice, rats, rabbits, sheep, goats, and chickens. Immunogens are delivered to the animal according to a predetermined schedule, and the animals are periodically bled, with successive bleeds having improved titer and specificity. The immunogens can be delivered to any antibody-producible site, for example, by intramuscular, intraperitoneal, subcutaneous and intravenous injections. Adjuvant may also be employed to enhance antibody production. Adjuvants may provide for sustained release of the injected immunogen, serve as a vehicle to help deliver the immunogen to the spleen and/or lymph nodes, and/or work to activate the various cells involved in the immune response, either directly or indirectly. Adjuvants may include, for example, Freund's Complete Adjuvant, Freund's Incomplete Adjuvant, Montanide ISA Adjuvants (Seppic, Paris, France), Ribi's

Adjuvants (Ribi Immuno Chem Research, Inc., Hamilton, MT), Hunter's TiterMax (CytRx Corp., Norcross, GA), Aluminum Salt Adjuvants, nitrocellulose-adsorbed protein, encapsulated antigens (such as liposome-entrapped antigen, nondegradable ethylene-vinyl acetate copolymer (EVAc)-entrapped antigen, and degradable polymer-entrapped antigen), and Gerbu Adjuvant (Gerbu Biotechnik GmbH, Gaiberg, Germany/C-C Biotech, Poway, CA).

Antibody producing cells can be obtained by hyperimmunizing a host animal, such as a mouse, with the desired immunogen by the methods described herein. The host is then killed, usually several days after the final immunization, the spleen and/or lymph nodes cells collected, and the cells immortalized resulting in anti-Aβ monoclonal antibody-producing hybridomas. Immortalization may be carried out by any method known to those of skill in the art or provided herein. Methods of immortalization may include, for example, fusion with a myeloma cell fusion partner (Kohler and Milstein (1975) *Nature 256*:495-497), EBV transformation, and transformation with bare DNA, e.g., oncogenes or retroviruses, or any other method which provides for stable maintenance of the cell line and production of monoclonal antibodies such as those described in *Antibodies: A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory, 1988. An exemplary immortalization method utilized in the Examples provided herein is the fusion of mouse spleen cells with mouse thymocytes.

Hybridomas can then be cloned and screened for avidity. Antibody avidity is the functional affinity or combining strength of an antibody with its antigen and is related to both the affinity of the reaction between the epitopes and paratopes, and the valences or recognition sites of the antibody and antigen. Avidity can be viewed as the total binding strength of all of an antibody's binding sites together. Affinity of an antibody reflects the goodness of fit of an antigenic determinant to a single antigen-binding site and is independent of the number of sites. Methods of assaying for antibody binding affinity are well known to those of skill in the art. Affinity or binding strength is generally expressed as the affinity constant (K). The affinity constant, alternatively called an association constant (K_a), can be determined by measuring the concentration of free antigen required to fill half of the antigen-binding sites on the antibody. The reeiprocal

of the antigen concentration that produces half-maximal binding is equal to the affinity constant of the antibody for the antigen. The affinity constant can be determined by measuring the association or dissociation constant for an antibody. Association and dissociation constants can be determined, for example, using a competition ELISA.

The degree of recognition of an antibody for an antigen is related to the selectivity (or specificity) of an antibody. Selectivity is considered a measure of the functional ability of an antibody to discriminate between the target antigen and other, chemically similar structures. Methods of assaying for antibody binding selectivity are well known to those of skill in the art. Selectivity can be determined, for example, by comparing the binding affinity of the antibody for the target antigen with the binding affinity of the antibody for other chemically similar molecules. Positive clones producing antibodies with high affinity and selectivity for specific Aβ peptides of interest can thus be chosen.

The desired monoclonal antibodies can be produced by injecting the hybridoma cells selected for their ability to produce high avidity antibodies into mice or by growing them in culture. With in vivo production, hybridoma cells are injected intraperitoneally into syngeneic animals, such as, for example, BALB/c mice or SCID mice, and ascites fluid obtained and purified. In addition, a primer or adjuvant may be used, such as, for example, pristane (2,6,10,14-tetramethyl pentadecane) or incomplete Freund's adjuvant to suppress the immune system so that the growth of the hybridoma cells is not strongly impaired, and to prohibit toxic irritation which may lead to peritonitis and the secretion of serous fluid. Purification may be carried out using standard antibody purification techniques, such as, for example, affinity chromatography using Protein A or Protein G.

i. Aβ42-selective antibody

Particular embodiments of the methods provided herein for identifying or screening for agents that modulate Aβ levels include a step of identifying an agent that modulates the level of Aβ42 in a sample. In one embodiment, the step involves identifying an agent that selectively modulates the level of Aβ42 in a sample relative to Aβ40 and/or increasing the level of Aβ39. Thus, the practice of some of the methods provided herein involves the ability to detect a particular species of Aβ, such as Aβ42, and to distinguish it from other species (*e.g.*, from other Aβ forms that do not contain the

"42" carboxy terminus, such as Aβ40). Antibodies and fragments thereof selective or specific for Aβ42 are provided herein. Also provided are isolated antibodies selective or specific for Aβ42. Further provided are amino acid sequences and proteins that portions of the antibodies. Also provided are isolated proteins that are portions of the antibodies. In a particular embodiment, the antibody is a mouse antibody. In a particular embodiment, the antibody is a monoclonal antibody, such as, for example, a mouse monoclonal antibody. In one embodiment, the Aβ42-selective antibody is one generated against a peptide based on a mammalian Aβ amino acid sequence, including, for example, a human Aβ amino acid sequence. In a particular embodiment, the Aβ42-selective antibody is an IgG. In one embodiment, the antibody type is IgG2a kappa.

The Aβ42-selective antibodies provided herein bind Aβ42 with minimal to no binding of other Aβ forms, *e.g.*, Aβ1-40, Aβ1-11, 1-28, 1-38, and 1-39). In a particular embodiment, the Aβ42-selective antibody has at least 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity for Aβ42 relative to other forms of Aβ, and, in particular Aβ40. In one embodiment, the Aβ42-selective antibody has at least about 1000-fold specificity or selectivity for Aβ42 relative to Aβ40. The antibodies selective for Aβ42 provided herein have a high affinity for binding to Aβ42. In a particular embodiment, the antibody has an affinity constant for binding to Aβ42 of at least about 10^5 l/mol, 2×10^5 l/mol, 3×10^5 l/mol, 4×10^5 l/mol, 5×10^5 l/mol, 6×10^5 l/mol, 7×10^5 l/mol, 8×10^5 l/mol, 9×10^5 l/mol, 10^6 l/mol, 2×10^6 l/mol, 3×10^6 l/mol or 4×10^6 l/mol or more. In one embodiment, the antibody has an affinity constant for binding to Aβ42 of at least about 4×10^6 l/mol. In a particular embodiment, the Aβ42-selective antibody has an affinity constant for binding to Aβ42 of at least about 4×10^6 l/mol and at least about 1000-fold specificity or selectivity for Aβ42 relative to Aβ40.

In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids as set forth in SEQ ID NO: 12. In a particular embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-107 or 1-95 of SEQ ID NO: 12. In another embodiment, an antibody or portion or fragment thereof contains a light chain variable region

containing the sequence of amino acids 1-95 of SEQ ID NO: 12. In a further embodiment, an antibody or portion or fragment thereof contains a light chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, or 1-94 of SEQ ID NO: 12. In a particular embodiment, the light chain is a kappa light chain. In another embodiment, the antibody or portion or fragment thereof that contains a sequence of amino acids set forth as amino acids 1-95 of SEQ ID NO: 12 further contains a joining (J) region. In a particular embodiment, the J region is a J_{kappa} region. The J region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In one embodiment, the J region contains a sequence of amino acids set forth as amino acids 96-107 as set forth in SEQ ID NO: 12. In one embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-95 of SEQ ID NO: 12 and a sequence of amino acids of a constant (C) region, such as, for example, a light chain C region. The C region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the C region is a C_{kappa} region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NOs: 63, 65 or 81. In a particular embodiment the antibody or fragment thereof contains a sequence of amino acids as set forth in SEQ ID NO: 97.

In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids set forth in SEQ ID NO: 14. In a particular embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-118 or 1-97 of SEQ ID NO: 14. In one embodiment, an antibody or portion or fragment thereof contains a heavy chain variable region containing the sequence of amino acids 1-97 of SEQ ID NO: 14. In a further embodiment, an antibody or portion or fragment thereof contains a heavy chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, or 1-96 of SEQ ID NO: 14. In a particular embodiment, the heavy chain is a γ heavy chain. In one embodiment, the antibody is an IgG_{2a}. In another embodiment, the antibody or portion or fragment thereof that contains a sequence of amino acids set forth

as amino acids 1-97 of SEQ ID NO: 14 further contains a diversity and joining ("DJ") region. The DJ region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the DJ region is a heavy chain DJ region, such as a DJ_γ region. In one embodiment, the DJ region contains a sequence of amino acids set forth as amino acids 98-118 as set forth in SEQ ID NO: 14. In one embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-97 of SEQ ID NO: 14 and a sequence of amino acids of a constant (C) region, such as, for example, a heavy chain C region. The C region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the C region is a C_γ region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular embodiment the antibody or fragment thereof contains a sequence of amino acids as set forth in SEQ ID NO: 98.

In one embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of SEQ ID NO: 12) and the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14). In another embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of SEQ ID NO: 12) and the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14) and further contains amino acid sequence of one or more J and/or DJ regions. For example, the J region can be a light and/or heavy chain J region. The J and/or DJ region(s) can be from any species, including, but not limited to mammals, such as, for example, primates, rodents and humans. Exemplary J regions include, but are not limited to, a J_{kappa} region (*e.g.*, such as one containing a sequence of amino acids 96-107 as set forth in SEQ ID NO: 12) and/or a heavy chain DJ region, such as a DJ_γ region (*e.g.*, such as one containing a sequence of amino acids 98-118 as set forth in SEQ ID NO: 14). Other exemplary J regions include, but are not limited to, a light chain J region (*e.g.*, such as one containing a sequence of amino acids set forth in SEQ ID NO: 46, 48, 50, 52, 54, 55,

57, 59, 61, 73, 75, 77, or 79 and/or a heavy chain J region (*e.g.*, such as one containing a sequence of amino acids set forth in SEQ ID NO: 67, 89, or 91). In another embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of SEQ ID NO: 12) and the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14) and further contains amino acid sequence of one or more constant regions. For example, the constant region can be a light and/or heavy chain constant region. The C region(s) can be from any species, including, but not limited to mammals, such as, for example, primates, rodents and humans. Exemplary constant regions include, but are not limited to, a C_{kappa} region. Exemplary light chain constant regions may contain a sequence of amino acids set forth in SEQ ID NO: 63, 65 or 81. Exemplary constant regions may also include, but are not limited to, a C_{γ} region. Exemplary heavy chain constant regions may contain a sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular embodiment of any of the antibodies, the antibody or portion or fragment thereof is an IgG_{2a} type.

Also provided are derivatives and modified immunoglobulins that have the capacity to bind to A*β*. In a particular embodiment, such molecules include fragments, such as Fab' or Fab'2 produced, for example, by the proteolytic cleavage of the mAb. Such molecules may also include single-chain immunoglobulins producible, for example, via recombinant means, such as Fv, scFv. Portions or fragments of antibodies include fragments that contain at least a portion of the antigen-binding region of the antibody. The portion of the antigen-binding region can be one that binds to the same antigenic determinant as the antibody with an affinity of at least about 1%, 5%, 10%, 15%, 20%, 25%, 50%, 60%, 70%, 75%, 80%, 90% or 100% of the affinity of the entire antibody. In particular embodiments, such fragments can be combined with one another (e.g., to form a diabody) or with other antibody fragments or receptor ligands to form "chimeric" binding molecules. Significantly, such chimeric molecules may contain substituents capable of binding to different epitopes of the same molecule (*e.g.*, two different A*β* epitopes). Whole antibodies molecules are large proteins, ~150 kDa in size, made up of four chains, two heavy chains (~50 kDa each), and two light chains (~25 kDa each). The

domains responsible for targeting specifically foreign entities is called the Fv domains. The Fv domain contains a portion of a heavy chain domain (HFv) and a light domain (LFv). Fv's are not produced by the body but can be engineered. An scFv fragment is an entity very similar to the Fv fragment, except the heavy and light chains are connected via a linker sequence. A dimer of scFv fragments is called a diabody. Fab fragments contain portions of heavy and light domains that are chemically linked. Fab fragments can be prepared from the parent antibody, by simple enzymatic hydrolysis. Thus, a "portion or fragment" of antibody refers to any of these aforementioned antibody fragments as well as to any fragment or portion of an antibody that retains an at least 100-fold, 200-fold, 300-fold, 400-fold, 500-fold up to 1000-fold selectivity for Aβ42 relative to other Aβ peptides, and particularly relative to Aβ40.

Also provided herein are nucleic acids encoding an antibody or a portion or fragment thereof. Further provided are isolated nucleic acids containing nucleotide sequences encoding portions of the antibodies. In a particular embodiment, the antibody is a mouse antibody. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO: 12. In a particular embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth as amino acids 1-107 or 1-95 of SEQ ID NO: 12. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes a light chain variable region containing the sequence of amino acids 1-95 of SEQ ID NO: 12. In a further embodiment, a nucleic acid contains a sequence of nucleotides that encodes a light chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, or 1-94 of SEQ ID NO: 12. In a particular embodiment, the light chain is a kappa chain. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-95 of SEQ ID NO: 12 and a J region. The J region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. For example, the J can contain the sequence of amino acids set forth in SEQ ID NO: 46, 48, 50, 52, 54, 55, 57, 59, 61, 73, 75, 77 or 79. In a particular embodiment, the J region contains a sequence of amino acids 96-107 as set forth in SEQ ID NO: 12. In another embodiment,

a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-95 of SEQ ID NO: 12 and a constant (C) region. The C region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. In particular embodiments, the C region is a light chain C region. For example, the C region can be a kappa light chain constant sequence. In one example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 63, 65 or 81. In another embodiment, a nucleic acid can further contain, in addition to a sequence of nucleotides encoding a C region, a sequence of nucleotides encoding a variable region such as those described herein above. In a particular embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 11 or the sequence of nucleotides 1-285 set forth in SEQ ID NO: 11.

In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO: 14. In a particular embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth as amino acids 1-118 or 1-97 of SEQ ID NO: 14. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes a heavy chain variable region containing the sequence of amino acids 1-97 of SEQ ID NO: 14. In a further embodiment, a nucleic acid contains a sequence of nucleotides that encodes a heavy chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, or 1-96 of SEQ ID NO: 14. In a particular embodiment, the heavy chain is an IgG_{2a} heavy chain. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-97 of SEQ ID NO: 14 and a DJ region. The DJ region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. For example, the DJ can contain the sequence of amino acids set forth in SEQ ID NO: 67, 89 or 91. In a particular embodiment, the DJ region contains a sequence of amino acids corresponding to 98 through 118 as set forth in SEQ ID NO: 14. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-97 of SEQ ID NO: 14 and one or more a constant (C) regions. The C region can be from any species, including but not limited to mammals, such as, for

example, primates, rodents and humans. In particular embodiments, the C region is a heavy chain C region. For example, the C region can be a heavy chain C_γ region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular example, the C region can be an IgG_{2a} heavy chain constant sequence. In another embodiment, a nucleic acid can further contain, in addition to a sequence of nucleotides encoding a C region, a sequence of nucleotides encoding a variable region such as those described herein above. In a particular embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 13 or the sequence of nucleotides 1-291 of SEQ ID NO: 13.

Nucleic acid constructs, including, for example, plasmids and expression vectors, are also provided herein. In one embodiment of a nucleic acid construct provided herein, the nucleic acid contains a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of SEQ ID NO: 12) and the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14). In a another embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 11 and SEQ ID NO: 13. In another embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 11 and SEQ ID NO: 13 or a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of SEQ ID NO: 12) and a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14) and further contains one or more sequences of nucleotides encoding one or more of the following amino acid sequences: a J region, *e.g.*, a light or a heavy chain J region, including, for example, a kappa light chain J region and a γ heavy chain J region, and a C region, *e.g.*, a light chain or heavy chain constant region, including, for example, a kappa light chain constant region, and a γ heavy chain C region, such as an IgG_{2a} heavy chain constant region. These regions can be from any species, including, but not limited to, mammals, such as, for example, primates, rodents and humans. Exemplary amino acid sequences of such regions can be any of those described herein above or known in the art.

Antibodies selective or specific for $A\beta42$ can be made by immunizing an animal

(*e.g.*, a mouse) with a peptide that contains a sequence of amino acids within the sequence of Aβ1-42 (such as human Aβ1-42; see, *e.g.*, SEQ ID NO: 4 amino acids 1-42) that includes amino acids C-terminal to amino acid 40 of Aβ. In a particular example, to minimize the likelihood of cross-reactivity of a generated antibody with the predominant Aβ40 species, a minimal peptidyl sequence of C-MVGGVVIA was used to immunize animals, which represents the Aβ35-42 region (*e.g.*, amino acids 35-42 of Aβ; see amino acids 35-42 of SEQ ID NO: 4). An N-terminal cysteine can be added for conjugation to an immunogenic carrier such as, for example, ovalbumin as described in Example 1. In a particular embodiment, an Aβ42-selective antibody provided herein is the monoclonal antibody A387 (described in detail in Examples 1 and 4). Antibody A387 demonstrates very high affinity for Aβ42 with a measured affinity constant of >4 X 10^6 l/mol. Furthermore, A387 has at least 1000-fold specificity for binding to Aβ42 versus Aβ40. Additionally, this antibody was shown to be highly selective for Aβ42 versus other AB peptides. When tested by ELISA methods, the A387 antibody showed no reactivity to Aβ1-11, 1-28, 1-38, and 1-39 peptides. The exceptionally high affinity and selectivity of the Aβ42-selective antibodies provided herein makes them a highly effective tool for detecting and quantitatively measuring Aβ42 and distinguishing this form of Aβ from other Aβ forms. Additionally, the Aβ42-selective antibodies provided herein are particularly useful for specifically assaying samples that contain detergents (such as Triton X-100, CHAPS, SHAPSO, Tween-2, and the like) or metal chelators (EDTA, EGTA, and the like) for Aβ42.

Antibodies provided herein can also be produced using recombinant DNA methods. For example, the recombinant production of immunoglobulin molecules, including humanized antibodies are described in U.S. Pat. Nos. 4,816,397 (Boss et al.), 4,816,567 (Cabilly et al.) U.K. patent GB 2,188,638 (Winter et al), and U.K. patent GB 2,209,757. Techniques for the recombinant expression of immunoglobulins, including humanized immunoglobulins, can also be found, among other places in Goeddel et al, Gene Expression Technology Methods in Enzymology Vol. 185 Academic Press (1991), and Borreback, Antibody Engineering, W. H. Freeman (1992). Additional information concerning the generation, design and expression of recombinant antibodies can be found

in Mayforth, Designing Antibodies, Academic Press, San Diego (1993).

The host cell used to express the recombinant antibodies provided herein may be either a bacterial cell, such as *Escherichia coli*, or a eukaryotic cell, such as a chinese hamster ovary cell. The choice of expression vector is dependent upon the choice of host cell, and may be selected so as to have the desired expression and regulatory characteristics in the selected host cell. The general methods for construction of the vector, transfection of cells to produce the host cell, culture of cells to produce the antibody are all well known in the art. Likewise, once produced, the recombinant antibodies may be purified by standard procedures of the art, including cross-flow filtration, ammonium sulphate precipitation, affinity column chromatography, gel electrophoresis and the like.

Antibodies can be made by constructing a vector containing a nucleic acid encoding a V region. Exemplary V regions include any of those described herein. The V region can be fused with a J region. The J region can be, for example, a light chain J region or a heavy chain J region, including, for example, a kappa light chain J region and a γ heavy chain J region. These regions can be from any species, including, but not limited to, mammals, such as, for example, primates, rodents and humans. Exemplary J regions include, but are not limited to, a J_{kappa} region (*e.g.*, such as one containing a sequence of amino acids 96-107 as set forth in SEQ ID NO: 12 or a sequence of amino acids 101-112 as set forth in SEQ ID NO: 16) and/or a heavy chain DJ region, such as a DJ_{γ} region (*e.g.*, such as one containing a sequence of amino acids 98-118 as set forth in SEQ ID NO: 14 or a sequence of amino acids 99-114 as set forth in SEQ ID NO: 18). Other exemplary J regions include, but are not limited to, human and mouse light chain J regions (*e.g.*, such as the ones containing a sequence of amino acids set forth in SEQ ID NOS. 73, 75, 77 or 79, and SEQ ID NOS. 46, 48, 50, 52, 54, 55, 57, 59 or 61 respectively) and human and mouse heavy chain J region (*e.g.*, such as the ones containing a sequence of amino acids set forth in SEQ ID NOS. 89 or 91, and SEQ ID NO. 67 respectively). In constructing the vector the nucleic acid encoding the V and J regions can further be fused with nucleic acid encoding a C region. The C region can be, for example, a light chain C region or a heavy chain C region, including, for example, a

kappa light chain constant region, and a γ heavy chain C region, such as an IgG_{2a} heavy chain constant region. These regions can be from any species, including, but not limited to, mammals, such as, for example, primates, rodents and humans. For example, mouse and human light chain C regions may contain a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO. 63 or 65 and SEQ ID NO 81, respectively. Mouse and human heavy chain C regions may contain a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO. 69 or 71 and SEQ ID NO 83, 85 or 87, respectively.

In certain embodiments, the recombinant antibodies provided herein may comprise a complete antibody molecule having full length heavy and light chains, or any fragment thereof, such as the Fab or $(Fab')_2$ fragments, a heavy chain and light chain dimer, or any minimal fragment thereof such as a Fv, an SCA (single chain antibody), and the like, specific for the particular Aβ antigen molecule.

The term humanized immunoglobulin or humanized antibody refers to an immunoglobulin comprising portions of immunoglobulins of different origin, wherein at least one portion is of human origin. Accordingly, provided herein are humanized immunoglobulins which bind to a mammalian Aβ peptide (e.g., human Aβ42 or Aβ40), said immunoglobulin comprising an antigen-binding region of nonhuman origin (e.g., rodent) and at least a portion of an immunoglobulin of human origin (e.g., a human framework region, a human constant region or portion thereof). For example, the humanized antibody can comprise portions derived from an immunoglobulin of nonhuman origin with the requisite specificity, such as a mouse, and from immunoglobulin sequences of human origin (e.g., a chimeric immunoglobulin), joined together chemically by conventional techniques (e.g., synthetic) or prepared as a contiguous polypeptide using genetic engineering techniques (e.g., DNA encoding the protein portions of the chimeric antibody can be expressed to produce a contiguous polypeptide chain).

Another example of the humanized immunoglobulins provided herein is an immunoglobulin containing one or more immunoglobulin chains comprising a CDR of nonhuman origin (e.g., one or more CDRs derived from an antibody of nonhuman origin)

and a framework region derived from a light and/or heavy chain of human origin (e.g., CDR-grafted antibodies with or without framework changes). In one embodiment, the humanized immunoglobulins can compete with murine A387 or B436 monoclonal antibodies for binding to the respective human Aβ peptides. In a particular embodiment, the antigen-binding region of the humanized immunoglobulin (a) is derived from A387 monoclonal antibody (e.g., as in a humanized immunoglobulin comprising CDR1, CDR2 and CDR3 of the A387 light chain and CDR1, CDR2 and CDR3 of the A387 heavy chain) or (b) is derived from B436 monoclonal antibody (e.g., as in a humanized immunoglobulin comprising CDR1, CDR2 and CDR3 of the B436 light chain and CDR1, CDR2 and CDR3 of the B436 heavy chain). Chimeric or CDR-grafted single chain antibodies are also encompassed by the term humanized immunoglobulin.

As set forth above, such humanized immunoglobulins can be produced using synthetic and/or recombinant nucleic acids to prepare genes (e.g., cDNA) encoding the desired humanized chain. For example, nucleic acid (e.g., DNA) sequences coding for humanized variable regions can be constructed using PCR mutagenesis methods to alter DNA sequences encoding a human or humanized chain, such as a DNA template from a previously humanized variable region (see e.g., Kamman, M., et al., Nucl. Acids Res., 17: 5404 (1989)); Sato, K., et al., Cancer Research, 53: 851-856 (1993); Daugherty, B. L. et al., Nucleic Acids Res., 19(9). 2471-2476 (1991); and Lewis, A. P. and J. S. Crowe, Gene, 101: 297-302 (1991)). Using these or other suitable methods, variants can also be readily produced. In one embodiment, cloned variable regions can be mutagenized, and sequences encoding variants with the desired specificity can be selected (e.g., from a phage library; see e.g., Krebber et al., U.S. Pat. No. 5,514,548; Hoogenboom et al., WO 93/06213, published Apr. 1, 1993)).

In certain embodiments, the humanized antibodies provided herein may comprise a complete antibody molecule having full length heavy and light chains, or any fragment thereof, such as the Fab or $(Fab')_2$ fragments, a heavy chain and light chain dimer, or any minimal fragment thereof such as a Fv, an SCA (single chain antibody), and the like, specific for the particular Aβ antigen molecule.

In one embodiment, an antibody or portion or fragment thereof provided herein

contains a sequence of amino acids as set forth in SEQ ID NO: 12 and/or SEQ ID NO: 14 (or portions thereof such as amino acids 1-95 of SEQ ID NO: 12 and/or amino acids 1-97 of SEQ ID NO: 14) or modifications thereof that retain the antigen-binding properties of an antibody containing one or both of these sequences of amino acids. Such modifications can be determined empirically and include, for example, conservative amino acid substitutions as well as deletions and additions of residues that do not substantially alter the antigen-binding properties. Determination of residues that do not substantially alter antigen binding properties can be accomplished empirically, such as by systematic replacement of each residue in the polypeptide with another amino acid, such as alanine, serine or glycine, and testing of the resulting polypeptide for its ability to bind to the antigen compared to the unmodified polypeptide. Those that retain at least 1, 10, 25, or 50% of the binding affinity compared to the unmodified polypeptide or that have an affinity constant of at least 10^6 are identified. Also polypeptides that include a portion of SEQ ID NO: 12, 14, 16, or 18 and retain such ability and modification thereof are included.

Also provided herein are methods for detecting Aβ42 and/or measuring Aβ42 levels or determining the Aβ42 content of a sample. The methods use antibodies provided herein. In one embodiment, the method includes steps of contacting a sample with an antibody or portion or fragment thereof provided herein and determining if the antibody (or portion or fragment thereof) forms any complexes with or binds to any molecules in the sample. The contacting can be performed under conditions whereby the antibody (or portion or fragment thereof) binds to or forms a complex with Aβ. In a particular embodiment, the antibody is selective for Aβ42 relative to other forms of Aβ, including Aβ1-11, 1-28, 1-38, 1-39 and 1-40. In one embodiment, the antibody is selective for Aβ42 relative to Aβ40. In other embodiments, the antibody or portion or fragment thereof is any one of the compositions as set forth herein above or described anywhere herein, including the Examples.

Specific immunoassay-related techniques and procedures that may be used in the methods for detecting Aβ42 and/or measuring Aβ42 levels or determining the Aβ42 content of a sample are described herein or known in the art. Any such procedures may

be employed in the methods. Exemplary formats include, but are not limited to, ELISA, sandwich assays, competitive immunoassays, radioimmunoassays, Western blots and indirect immunofluorescent assays. In a particular embodiment of the methods for detecting Aβ42 and/or measuring Aβ42 levels or determining the Aβ42 content of a sample provided herein, an Aβ42-selective antibody or portion or fragment thereof provided herein is contacted with the sample, and binding between the antibody (or portion or fragment thereof) and any protein or peptide in the sample is assessed in a sandwich assay, as described herein.

ii. Aβ1-12 antibody

Antibodies that react substantially similarly to any Aβ peptide which contains an amino-terminal sequence substantially as set forth in the sequence of amino acids 1-12 of SEQ ID NO: 4 are also provided herein. Also provided are isolated proteins that are portions of the antibodies. Included among such antibodies are antibodies referred to herein as Aβ1-12 antibodies. Such antibodies can be used, for example, in immunoassays to detect all forms of Aβ (total Aβ), or at least all forms of Aβ containing the amino-terminus as set forth in amino acids 1-12 of SEQ ID NO: 4. Such antibodies can also be used in conjunction with antibodies that are selective for a particular type or types of Aβ, *e.g.*, Aβ42 (including Aβ42-selective antibodies provided herein), for example, to determine the ratio of Aβ42 to total Aβ in a sample. Antibodies that react substantially similarly to any Aβ peptide can also be used as capture or detection antibodies in conjunction with selective antibodies in sandwich immunoassays to detect a particular form of Aβ, *e.g.*, Aβ42. Such methods using the Aβ1-12 antibodies and Aβ42-selective antibodies provided herein are described herein.

In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids as set forth in SEQ ID NO: 16. In a particular embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-112 or 1 to 100 of SEQ ID NO: 16. In one embodiment, an antibody or portion or fragment thereof contains a light chain variable region containing the sequence of amino acids 1-100 of SEQ ID NO: 16. In a further embodiment, an antibody or portion or fragment thereof contains a light chain variable

region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-95, 1-96, 1-97, 1-98, or 1-99 of SEQ ID NO: 16. In a particular embodiment, the light chain is a kappa light chain. In another embodiment, the antibody or portion or fragment thereof that contains a sequence of amino acids set forth as amino acids 1-100 of SEQ ID NO: 16 further contains a joining (J) region. In a particular embodiment, the J region is a J_{kappa} region. The J region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In one embodiment, the J region contains a sequence of amino acids set forth as amino acids 101-112 as set forth in SEQ ID NO: 16. In one embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-100 of SEQ ID NO: 16 and a sequence of amino acids of a constant (C) region, such as, for example, a light chain C region. The C region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the C region is a C_{kappa} region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NOs: 63, 65 or 81. In a particular embodiment the antibody or fragment thereof contains a sequence of amino acids as set forth in SEQ ID NO: 99.

In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids set forth in SEQ ID NO: 18. In a particular embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-114 or 1-98 of SEQ ID NO: 18. In one embodiment, an antibody or portion or fragment thereof contains a heavy chain variable region containing the sequence of amino acids 1-98 of SEQ ID NO: 18. In a further embodiment, an antibody or portion or fragment thereof contains a heavy chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96, or 1-97 of SEQ ID NO: 18. In a particular embodiment, the heavy chain is a γ heavy chain. In one embodiment, the antibody is an IgG_{2a}. In another embodiment, the antibody or portion or fragment thereof that contains a sequence of amino acids set forth as amino acids 1-98 of SEQ ID NO: 18 further contains a diversity and joining (DJ) region. The DJ region can be one from any species, including but not

limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the DJ region is a heavy chain DJ region, such as a DJ_γ region. In one embodiment, the DJ region contains a sequence of amino acids set forth as amino acids 99-114 as set forth in SEQ ID NO: 18. In one embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-98 of SEQ ID NO: 18 and a sequence of amino acids of a constant (C) region, such as, for example, a heavy chain C region. The C region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the C region is a C_γ region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular embodiment the antibody or fragment thereof contains a sequence of amino acids as set forth in SEQ ID NO: 100

In one embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and the sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18). In another embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and the sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18) and further contains amino acid sequence of one or more J regions. For example, the J region can be a light and/or heavy chain J region. The J and/or DJ region(s) can be from any species, including, but not limited to mammals, such as, for example, primates, rodents and humans. Exemplary J regions include, but are not limited to, a J_{kappa} region (*e.g.*, such as one containing a sequence of amino acids 101-112 as set forth in SEQ ID NO: 16) and/or a heavy chain DJ region, such as a DJ_γ region (*e.g.*, such as one containing a sequence of amino acids 99-114 as set forth in SEQ ID NO: 18). Other exemplary J regions include, but are not limited to, a light chain J region (*e.g.*, such as one containing a sequence of amino acids set forth in SEQ ID NO: 46, 48, 50, 52, 54, 55, 57, 59, 61, 73, 75, 77 , or 79) and/or a heavy chain J region (*e.g.*, such as one containing a sequence of amino acids set forth in SEQ ID NO: 67, 89 or 91). In another embodiment, an antibody or portion or

fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and the sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18) and further contains amino acid sequence of one or more constant regions. For example, the constant region can be a light and/or heavy chain constant region. The C region(s) can be from any species, including, but not limited to mammals, such as, for example, primates, rodents and humans. Exemplary constant regions include, but are not limited to, a C_{kappa} region. Exemplary light chain constant regions may contain a sequence of amino acids set forth in SEQ ID NO: 63, 65 or 81. Exemplary constant regions may also include, but are not limited to, a C_γ region. Exemplary heavy chain constant regions may contain a sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular embodiment of any of the antibodies, the antibody or portion or fragment thereof is an IgG_{2a} type.

In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids as set forth in SEQ ID SEQ ID NO: 16 and/or SEQ ID NO: 18 (or portions thereof such as amino acids 1-100 of SEQ ID NO: 16 and 1-98 of SEQ ID NO: 18) or modifications thereof that retain the antigen-binding properties of an antibody containing one or both of these sequences of amino acids. Such modifications can be determined empirically and include, for example, conservative amino acid substitutions as well as deletions and additions of residues that do not substantially alter the antigen-binding properties. Determination of residues that do not substantially alter antigen binding properties can be accomplished empirically, such as by systematic replacement of each residue in the polypeptide with another amino acid, such as alanine, serine or glycine, and testing of the resulting polypeptide for its ability to bind to the antigen compared to the unmodified polypeptide. Those that retain at least 1, 10, 25, or 50% of the binding affinity compared to the unmodified polypeptide or that have an affinity constant of at least 10^6 are identified. Also polypeptides that include a portion of SEQ ID NO: 16 or 18 and retain such ability and modification thereof are included.

Also provided herein are nucleic acids encoding an antibody or a portion or fragment thereof. Further provided are isolated nucleic acids containing nucleotide

sequences encoding portions of antibodies. In a particular embodiment, the antibody is a mouse antibody. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO: 16. In a particular embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth as amino acids 1-112 or 1-100 of SEQ ID NO: 16. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes a light chain variable region containing the sequence of amino acids 1-100 of SEQ ID NO: 16. In a further embodiment, a nucleic acid contains a sequence of nucleotides that encodes a light chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-95, 1-96, 1-97, 1-98, or 1-99 of SEQ ID NO: 16. In a particular embodiment, the light chain is a kappa chain. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-100 of SEQ ID NO: 16 and a J region. The J region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. For example, the J can contain the sequence of amino acids set forth in SEQ ID NO: 46, 48, 50, 52, 54, 55, 57, 59, 61, 73, 75, 77 or 79. In a particular embodiment, the J region contains a sequence of amino acids from 101 to 112 as set forth in SEQ ID NO: 16. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-100 of SEQ ID NO: 16 and a constant (C) region. The C region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. In particular embodiments, the C region is a light chain C region. For example, the C region can be a kappa light chain constant sequence. In one example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 63, 65 or 81. In another embodiment, a nucleic acid can further contain, in addition to a sequence of nucleotides encoding a C region, a sequence of nucleotides encoding a variable region such as those described herein above. In a particular embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 15 or the sequence of nucleotides 1-300 set forth in SEQ ID NO: 15.

In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO: 18. In a particular

embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth as amino acids 1-114 or 1-98 of SEQ ID NO: 18. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes a heavy chain variable region containing the sequence of amino acids 1-98 of SEQ ID NO: 18. In a further embodiment, a nucleic acid contains a sequence of nucleotides that encodes a heavy chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96, or 1-97 of SEQ ID NO: 18. In a particular embodiment, the heavy chain is an IgG_{2a} heavy chain. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-98 of SEQ ID NO: 18 and a DJ region. The DJ region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. For example, the DJ can contain the sequence of amino acids set forth in SEQ ID NO: 67, 89 or 91. In a particular embodiment, the DJ region contains a sequence of amino acids from 99 to 114 as set forth in SEQ ID NO: 18. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-98 of SEQ ID NO: 18 and one or more a constant (C) regions. The C region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. In particular embodiments, the C region is a heavy chain C region. For example, the C region can be a heavy chain C_γ region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular example, the C region can be an IgG_{2a} heavy chain constant sequence. In another embodiment, a nucleic acid can further contain, in addition to a sequence of nucleotides encoding a C region, a sequence of nucleotides encoding a variable region such as those described herein above. In a particular embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 17 or the sequence of nucleotides 1-294 set forth in SEQ ID NO:17.

Nucleic acid constructs, including, for example, plasmids and expression vectors, are also provided herein. In one embodiment of a nucleic acid construct provided herein, the nucleic acid contains a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and the

sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18). In a another embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 15 and SEQ ID NO: 17. In another embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 15 and SEQ ID NO: 17 or a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18) and further contains one or more sequences of nucleotides encoding one or more of the following amino acid sequences: a J region, *e.g.*, a light or a heavy chain J region, including, for example, a kappa light chain J region and a γ heavy chain J region, and a C region, *e.g.*, a light chain or heavy chain constant region, including, for example, a kappa light chain constant region, and a γ heavy chain C region, such as an IgG_{2a} heavy chain constant region. These regions can be from any species, including, but not limited to, mammals, such as, for example, primates, rodents and humans. Exemplary amino acid sequences of such regions can be any of those described herein above or known in the art.

Antibodies that bind substantially similarly to any Aβ peptide which contains an amino-terminal sequence substantially as set forth in the sequence of amino acids 1-12 of SEQ ID NO: 4 can be generated using animal immunization or recombinant DNA protocols described herein or known in the art. For example, such an antibody provided herein, referred to as B436 (see EXAMPLES 2 and 4), was generated by designing a peptide immunogen having the sequence DAEFRHDSGYEV-C that represents the Aβ1-12 region. The resulting murine monoclonal antibody was determined to have high titer for both Aβ40 and Aβ42 peptides.

iii. Aβ40 antibody

Antibodies that bind Aβ40 (*e.g.*, a form of Aβ containing the sequence of amino acids 1-40 of SEQ ID NO: 4) were also generated for use in methods described herein. For example, such antibodies can be used in particular embodiments of the methods for identifying agents that modulate Aβ42 levels. In these embodiments, which are described herein, a sample containing APP and/or a portion(s) thereof is contacted with a

test agent and an agent is identified that selectively modulates Aβ42 levels relative to Aβ40 levels. In a particular method, the Aβ42 levels of a sample are assessed using an Aβ42-selective antibody, such as provided and described herein, and the Aβ40 levels are assessed using an antibody that binds Aβ40.

An Aβ40 antibody was produced using animal immunization protocols as described herein. The Aβ40 antibody was prepared using the same protocol as described herein for production of antibody A387 (an Aβ42-selective antibody) production except that the peptide C-AIIGLMVGGVV (the sequence of amino acids 30-40 of SEQ ID NO: 4) was used to conjugate to ovalbumin and immunize mice. Subsequent titering was performed as described for the Aβ42-selective antibody.

(b) Aβ Assays

Immunoassays for detecting protein are well known to those of skill in the art. Exemplary immunoassay formats include ELISA, competitive immunoassays, radioimmunoassays, Western blots, indirect immunofluorescent assays, *in vivo* expression or immunization protocols with purified protein preparations. In general, an immunoassay to detect a protein or peptide involves contacting a cell-based or cell-free sample with the antibody of interest and incubating for a period of time sufficient to allow binding of antibody to the epitope, usually at least about 10 minutes. Detection of immunocomplex formation is well known in the art and may be achieved by methods generally based upon the detection of a label or marker, such as any of the radioactive, fluorescent, luminous, biological or enzymatic tags. Examples of the radioisotopes include 125I, 131I, 3H and 14C. Enzymatic tags that are stable and have a high specific activity are particularly suited for these methods. Examples of enzymatic tags include β-galactosidase, β-glucosidase, alkaline phosphatase, peroxidase, and malate dehydrogenase. Examples of fluorescent tags include fluorescamine and fluorescein isothiocyanate. Luminous tags include, for example, luminol, luminol derivatives, luciferin and lucigenin. Labels are well known to those skilled in the art (see U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241, each incorporated herein by reference).

A primary antibody may be directly labeled with radioisotopes, enzymes,

fluorescers, chemiluminescers, or other labels for direct detection. Alternatively, a secondary binding ligand such as a second antibody or a biotin/avidin ligand-binding arrangement may be used. The secondary ligand or reagent may be useful for amplifying the signal. Such reagents are well known in the art. For example, the primary antibody may be conjugated to biotin, with horseradish peroxidase-conjugated avidin added as a second stage reagent. Final detection uses a substrate that undergoes a color change in the presence of the peroxidase. The absence or presence of antibody binding may be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, scintillation counting, luminometer, etc.

Detection and measurement of Aβ peptides can involve the use of a two-site or "sandwich" assay employing two antibodies, one antibody capable of distinguishing an Aβ peptide (*e.g.*, Aβ42) from other Aβ peptides that might be found in the sample and a second antibody. One of the antibodies serves to capture the antigen while the other is used to detect the captured antigen or the antibody-antigen complex. Thus, for example, an antibody that is selective for a particular Aβ (*e.g.*, Aβ42) can be used as a capture antibody while an antibody that binds Aβ peptides either non-selectively or selectively, is used as a detection antibody. The first and second antibody reactions may be conducted simultaneously or sequentially. The detection antibody is conjugated to a detectable label as described above. In a particular embodiment, the detectable label is an enzymatic tag. In a further embodiment, the label is alkaline phosphatase and the presence or absence of antibody binding is determined by luminescence of a substrate that undergoes a color change in the presence of alkaline phosphatase, such as, for example, CDP-Star chemiluminescence substrate (Tropix, Inc.). Further, in the sandwich immunoassay methods, the Aβ selective antibodies or the antibodies used for labeling are not necessarily of one kind, but two or more kinds of antibodies may be used as mixtures for the purpose of enhancing the measuring sensitivity.

In an example of a method for detecting or measuring Aβ by the sandwich technique, the anti-Aβ antibody used in the first reaction can be reactive to a portion(s) of the Aβ peptide that is different from the portion(s) that the antibody used in the second reaction recognizes. For example, when the antibody used in the first reaction recognizes

a partial peptide on the C-terminal portion of Aβ, the antibody used in the second reaction generally is one that recognizes a partial peptide other than the partial peptide on the C-terminal portion (for example, a partial peptide on the N-terminal portion of Aβ).

In a particular embodiment of a method for detecting Aβ42 and/or measuring Aβ42 levels or determining the Aβ42 content of a sample provided herein, an Aβ42-selective antibody or portion or fragment thereof provided herein is used as the antibody of the first reaction in the sandwich assay (primary antibody). For example, the Aβ42-selective antibody, or portion or fragment thereof, can be one that contains the sequence of amino acids 1-95 of SEQ ID NO: 12 and/or the sequence of amino acids 1-97 of SEQ ID NO: 14. The secondary antibody can be any antibody that recognizes an epitope within the Aβ42 peptide. In one embodiment, the secondary antibody reacts with a portion(s) of Aβ42 that is different than the site(s) at which the primary antibody reacts. In a particular embodiment, the antibody of the second reaction in the sandwich assay (secondary antibody) is reactive with an N-terminal portion of Aβ42. The secondary antibody (or portion or fragment thereof) can be one that is reactive to more than one species of Aβ and can be reactive with most if not all forms of Aβ. In a particular embodiment, the secondary antibody (or portion or fragment thereof) is reactive with Aβ peptides containing amino acids 1-12 of SEQ ID NO: 4. For example, the secondary antibody, or portion or fragment thereof, can be one that contains the sequence of amino acids 1-100 of SEQ ID NO: 16 and/or the sequence of amino acids 1-98 of SEQ ID NO: 18. The secondary antibody can be used as the detection antibody and can be conjugated to a detectable label. In a particular embodiment, the detectable label is alkaline phosphatase and the presence or absence of antibody binding is determined by luminescence of a substrate that undergoes a color change in the presence of alkaline phosphatase, such as, for example, CDP-Star chemiluminescence substrate (Tropix, Inc.).

When a method for detecting Aβ42 and/or measuring Aβ42 levels or determining the Aβ42 content of a sample as provided herein is used as method for assessing the Aβ42 levels in a step of identifying an agent that selectively modulates Aβ42, it may be combined with a method for detecting and/or measuring Aβ40 in a sample, as described herein. In such methods for identifying agents that selectively modulate Aβ42 levels, the

Aβ42 level of one or more samples is assessed to identify an agent that modulate Aβ42 levels, and the Aβ40 level of one or more samples is assessed to identify those Aβ42-modulating agents that do not alter Aβ40 levels. One method for detecting and/or measuring Aβ40 in a sample for use in these methods is the above-described sandwich assay wherein an Aβ40-selective antibody (or portion or fragment thereof) is substituted for an Aβ42-selective antibody or portion or fragment thereof as the primary antibody. In a particular embodiment, the Aβ40-selective antibody is one that recognizes amino acids 30-40 of Aβ (for example, amino acids 30-40 of SEQ ID NO: 4), such as is described herein. For example, an Aβ40-selective antibody can be prepared by immunizing animals with the peptidyl sequence representing Aβ30-40 region, as described herein.

Sandwich ELISA-based assays such as these for use in methods for detecting Aβ42 and/or measuring Aβ42 levels or determining the Aβ42 content of a sample as provided herein can be performed in a microtiter plate format wherein the primary antibody is coated into the wells of the plate and the sample is added to the wells. After washing, the secondary antibody (which can be conjugated to a label such as alkaline phosphatase) is added to the wells which are washed prior to adding a substrate, *e.g.*, a chemiluminescent substrate, for detection of bound Aβ42. Such methods provide a large linear range, such as, for example, about 75-2000 pg/well, high dynamic range, *e.g.*, about 3-30 fold over background in linear range (signal:noise), low sensitivity limit, such as, for example, less than about 20 pg/well, and selectivity for Aβ42, *e.g.*, at least about 1000-fold selectivity for Aβ42 over other Aβ peptides, making the method highly amenable to high-throughput screening for agents that modulate Aβ42 levels.

Smaller Aβ peptides, for example, Aβ peptides having a C-terminal end that terminates before amino acid 40 (see, *e.g.*, the sequence of amino acids 1-40 of SEQ ID NO: 4) may also be detected in the methods provided herein. In particular embodiments, these peptides are measured by their mass, size, and/or charge. For example, peptides may be immunoprecipitated with an antibody reactive to the amino-terminal end of Aβ. For example, the anti-Aβ1-12 antibody described herein may be used for immunoprecipitation of these peptides. Immunoprecipitated peptides may then be identified by any method known to those of skill in the art including, for example,

electrophoresis and mass spectrometry. In a particular embodiment, cells expressing wild-type APP are treated with test agent or vehicle control for 18 h. Media is collected and immunoprecipitated using an anti-Aβ1-12:Sepharose® column for 4 h. Bound peptides are eluted with 0.1% TFA/50% acetonitrile and spotted onto NP2 CHIPS. Mass spectrometer analysis is performed on a PBS II Protein Chip Reader (Ciphergen). Data may be normalized to an internal standard, such as $A\beta_{1-11}$ that is spiked into the media prior to the immunoprecipitation.

All assays and procedures, including antibody-antigen reactions, generally can be conducted under conditions recognized by those of skill in the art as standard conditions.

d. Alterations of APP cleavage or processing, Aβ processing or Aβ levels

Methods for identifying or screening for agents that modulate Aβ levels can include a step of identifying an agent that alters cleavage (particularly the Aβ peptide-producing cleavage) of APP (and/or portion(s) thereof), processing or APP (and/or portion(s) thereof), Aβ processing and/or Aβ levels of a sample. The step of identifying an agent that alters such parameters that can affect Aβ levels typically involves making assessments of one or more of the parameters. As described herein, there are a number of ways in which APP cleavage, APP processing, Aβ processing and the Aβ levels of a sample can be assessed, including, but not limited to, immunoassays for detection and/or quantitation of one or more peptides, proteins and/or fragments thereof that are reflective of these parameters. A step of identifying an agent that alters one or more of these parameters can thus involve assessment of one or more of the parameters and a determination as to whether the parameter(s) is altered under a condition of the presence of the agent.

Determining if APP cleavage (particularly the Aβ peptide-producing cleavage), APP processing, Aβ processing and/or Aβ levels of a sample is altered by a test agent can involve comparing one or more of these parameters in the presence and absence of the test agent. Thus, in general, the agent identification step can involve a comparison of the cleavage (particularly the Aβ peptide-producing cleavage) of APP (and/or portion(s) thereof), processing or APP (and/or portion(s) thereof), Aβ processing and/or Aβ levels

of a sample that has been contacted with a test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the Aβ-producing cleavage or processing of APP (and/or portion(s) thereof), the processing of Aβ and/or the Aβ levels of the test and control samples differ, then the agent is identified as one that modulates the level of one or more Aβ peptides.

(1) Contacting sample with test agent

A sample for use in the methods of identifying or screening for agents that modulate Aβ levels as described herein can be maintained under conditions in which APP (and/or portion(s) thereof, including Aβ) can undergo cleavage and/or processing (*e.g.*, catabolism, degradation). If the sample is a test sample, it is contacted with a test agent. If the sample is a control sample, it can be one that is not contacted with test agent. Generally, a control sample is substantially similar to the test sample and maintained under substantially similar conditions as the test sample, but is not contacted with test agent. A control sample can be the same physical sample as the test sample (*e.g.*, prior to addition of test agent) or can be a different sample.

Depending on the type of control (*e.g.*, reference control, negative control or positive control), a control sample may be manipulated in various ways. For example, if a control sample is a vehicle control, it may be contacted with a "vehicle", such as a medium, or element thereof, in which the test agent is contained, but that lacks the test agent. Examples of such "vehicles" include suspension, solubilizing reagents, emulsifiers, and compositions that generally serve to facilitate retention and administration of a test agent. In a particular example, a vehicle control can be DMSO. A positive control can be a sample that has been treated using known processes/compositions to achieve an effect that is desired by a test agent that is a "positive" identified as one that modulates Aβ levels. Thus, for example, if the methods are conducted with the specific purpose of identifying an agent that reduces one or more Aβ levels, then a positive control sample could be one that is treated with an agent known to reduce Aβ levels. One particular example is an APP-containing sample that has been contacted with a β- and/or γ-secretase inhibitor, such as, for example, DAPT.

Test samples can be treated with a range of doses or concentrations of the test

agent or with only a single concentration of agent. When a range of different test agent concentrations is used in contacting a plurality of samples in parallel and compared to the magnitude of any effect each different concentration may have on the parameter(s) (*e.g.*, Aβ level of samples) being assessed (*e.g.*, a dose-response study), a more detailed analysis and profile of the test agent can be made. For example, it may be possible to determine values such as EC_{50} or IC_{50} for the test agent to estimate the potency of an agent. The methods provided herein allow for the identification of very potent Aβ-modulating agents. Particular embodiments of the methods provide for the identification of agents with an EC_{50} or IC_{50} for modulating (*e.g.*, increasing or decreasing) Aβ levels of 100 μM, 75 μM, 50 μM, 40 μM, 30 μM, 25 μM, 20 μM, 15 μM, or 10 μM or lower. In a particular embodiment of the methods, agents are identified that have an EC_{50} or IC_{50} for modulating (*e.g.*, increasing or decreasing) Aβ levels of less than about 25 μM. In a further embodiment of the methods, agents are identified having an EC_{50} or IC_{50} of less than about 20 μM. In one particular embodiment, agents are identified that have such values for an IC_{50} for reducing the levels of Aβ42. Generally, such a more detailed analysis is conducted after a test agent has been identified as one that alters one or more of the parameters at a threshold or test concentration, such as is typically done in a high-throughput screening of test agents. Threshold or test concentrations can be, for example, about 1 μM, 2 μM, 5 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 35 μM, 40 μM, 45 μM, 50 μM, 75 μM, 100 μM or more. In a one example, the threshold or test concentration can be less than about 50 μM, 40 μM, 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. In a particular example, the threshold or test concentration can be less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. Generally, by screening at a lower test concentration, the agents identified as modulators of Aβ may tend to be more potent than if they had been identified at a higher test concentration.

A sample for use in the methods of identifying or screening for agents that modulate Aβ levels as described herein can be maintained under conditions in which APP (and/or portion(s) thereof, including Aβ) can undergo cleavage and/or processing (*e.g.*, catabolism, degradation) for an appropriate amount of time prior to being used in the methods of identifying Aβ-modulating agents and after being contacted with test

agent. Such time periods can be empirically determined and generally are such to allow for detectable levels of APP cleavage or processing and/or Aβ formation or processing to occur. Similarly, the sample is contacted with test agent for an appropriate amount of time or range of time periods. Typically, when the methods are being practiced in a high-throughput screening format, a single time period of contacting is used. In one example, the time period can be on the order of minutes to hours depending, in part, on the type of sample, *e.g.*, intact cells or cell-free medium.

In one particular example of a method for identifying an agent that modulates Aβ levels, cell cultures capable of APP expression and processing (*e.g.*, CHO cells transfected with DNA encoding human APP695 and human PS1) are plated in the wells of a microtiter plate and allowed to adhere for about 24 hours. The separate samples in the wells were then either treated or not treated with a test agent (~30 μM). Samples treated with DMSO vehicle (0.12%) alone were a negative control. Samples treated with 1 μM DAPT for 18 hours were used as a positive control. Supernatant removed from the wells was analyzed in a sandwich ELISA to assess the level of Aβ42 in each sample. The ELISA was conducted in a microtiter plate format using an Aβ42-selective monoclonal antibody provided herein (antibody A387) as a capture antibody which was incubated with supernatant for 1 hour. After washing of the plate, the wells were incubated for 2 hours with a detection antibody generated against an Aβ1-12 peptide, as described herein, and conjugated to alkaline phosphatase. A chemiluminescence substrate was added to the wells and, after 30 minutes, the luminescence was quantified to assess and compare Aβ42 levels of the test and control samples in order to determine any differences and identify agents that modulate Aβ (and in particular Aβ42) levels.

(2) Evaluating alterations

In conducting the methods of identifying an agent that modulates Aβ levels, a way in which an agent can be identified is by identifying an agent that alters the cleavage or processing of APP (and/or portion(s) thereof), the processing of Aβ and/or Aβ levels. An alteration can be, for example, any detectable difference in the cleavage or processing of APP (and/or portion(s) thereof), the processing of Aβ and/or Aβ levels of a sample that has been contacted with a test agent as compared to a sample that has not been

contacted with the test agent. Methods for assessing the cleavage or processing of APP (and/or portion(s) thereof), the processing of Aβ and Aβ levels are described and provided herein and additional assessment methods are known in the art. Thus, any difference in any one or more of these processes or compositions as detected in the assessment of test and control samples can be an alteration by which an Aβ-modulating agent can be identified.

The extent of the difference can vary depending on a variety of factors, including, for example, the particular parameter being assessed and compared, the assessment method used and the conditions under which the assessment was conducted, the concentration of the test agent used as well as other factors. Thus, for example, the difference may be an about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or more than 75% difference in the assessed parameter, *e.g.*, a level or amount of composition, activity or processing, when compared under differing conditions, *e.g.*, in the presence and absence of a test agent. In one example of a particular high-throughput format of the methods, a test agent was identified as an agent that modulates Aβ42 levels if there was a greater than about 50% difference in the Aβ42 levels of test and control samples. In a particular example, a test agent was identified as an agent that reduces Aβ42 levels if the Aβ42 level of a test sample was more than about 50% lower than the Aβ42 level of a control sample.

e. Assessment of selectivity of Aβ-modulating agents

Cellular and extracellular Aβ levels are governed by numerous mechanisms and activities involved in Aβ synthesis through APP processing and in Aβ catabolism, degradation and clearance. These mechanisms include multiple components, such as, for example, enzymes and facilitator proteins, many of which have multiple substrates and/or multiple, closely related protein family members. In addition, some of the enzymes, *e.g.*, γ-secretase, may function as a part of a complex that includes a number of other proteases. Although any of these components and mechanisms, individually or in combination, are potential targets for modulation in order to ultimately modulate Aβ levels, modulation of these targets may also affect other processes (i.e., other than the processing of APP and/or Aβ) and the levels of other molecules due to the multiplicity of

component function and relatedness and interaction of some components to non-eomponent molecules. Modulation of Aβ levels that also involves modulation of other cellular processes and elements, i.e., non-specific modulation of Aβ levels, can result in undesired side effects. Methods of identifying agents that more specifically or selectively modulate Aβ levels are provided herein. The methods can be used to identify agents that selectively modulate the levels of one or more Aβ peptides without substantially affecting compositions and mechanisms that are not significantly involved in the generation, degradation and/or clearance of one or more Aβ peptides.

Using the methods provided herein, agents identified as Aβ-modulating agents can also be profiled with respect to the specificity or selectivity of their modulation.

(1) Assessment of Aβ peptide selectivity

Cleavage of APP to generate Aβ yields a number of Aβ peptides that can differ at the C-terminus, *e.g.*, Aβ1-43, Aβ1-42, Aβ1-40, and others. The C-terminal heterogeneity is the result of cleavage by distinct activities of γ-secretase and/or multiple γ-secretases. An agent that modulates the levels of all or most or more than one or two Aβ peptides may be non-selectively modulating components and mechanisms involved in processes other than the generation or degradation of Aβ in addition to modulating components and mechanisms of Aβ synthesis and degradation. Agents that selectively modulate the levels of one or two Aβ peptides, or a particular subset of Aβ peptides, are less likely to affect other compositions, activities and mechanisms and are therefore desired. Agents that selectively modulate the level of Aβ42 are of particular interest because Aβ42 is one of the predominant forms found in amyloid plaques, and is deposited early and selectively in the cerebral cortex of brains of individuals harboring some FAD-linked mutations. Aβ42 formation is also selectively elevated in some FAD-linked mutations.

Methods are provided herein for identifying or screening for an agent that selectively modulates Aβ levels. In one embodiment, the method identifies agents that alter the level of a particular form or forms of Aβ to a greater extent than they alter the levels of one or more other forms of Aβ. In a particular embodiment, such agents alter the level of a particular form or forms of Aβ without substantially affecting or altering the level of one or more other Aβ peptides. In one embodiment of the methods, an agent

that selectively modulates Aβ42 levels is identified. The agent can, for instance, selectively modulate Aβ42 levels relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In a particular embodiment, the agent modulates the levels of Aβ42 and Aβ39 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In a particular embodiment of the method, compounds are identified that selectively modulate Aβ peptides having a C-terminal end that terminates before amino acid 40. In a particular embodiment compounds are identified that selectively modulate the level of Aβ39. In a particular embodiment, the methods identify an agent that selectively increases Aβ39 levels. In another embodiment the methods identify an agent that selectively decreases Aβ39 levels.

In general, the methods of identifying or screening for an agent that selectively modulates the level of an Aβ peptide relative to one or more other Aβ peptides includes steps of contacting a sample containing APP and/or a portion(s) thereof (including, for example one or more Aβ peptides) with a test agent and identifying an agent that alters the level of an Aβ peptide to a greater extent than it alters the level of one or more other Aβ peptides. The process of identifying an agent that modulates the level of an Aβ peptide in a sample can be carried out in a number of ways as described herein. For example, the Aβ peptide level in a sample that has been contacted with a test agent (test sample) can be compared with the Aβ peptide level in a sample that has not been contacted with the test agent (control sample). If the Aβ peptide levels in the two samples differ, then the agent is identified as one that modulates the level of the Aβ peptide. Methods for assessing the level of a particular Aβ peptide in a sample are described herein or known in the art. Such methods include, but are not limited to, immunoassays employing peptide-specific antibodies, mass spectrometry and electrophoretic analyses.

In a particular embodiment of the methods for identifying or screening for an agent that selectively modulates the level of an Aβ peptide, the Aβ42 levels of samples are assessed by contacting a sample with an antibody (or portion or fragment thereof) that selectively binds to Aβ42 (*e.g.*, the sequence of amino acids 1-42 of SEQ ID NO: 4). In one embodiment, the antibody is any one of the Aβ42-selective antibodies provided

herein, such as, for example, an antibody that contains the sequence of amino acids 1 to about 95, 94, 93, 92, 91, 90, 85, 80, 70, 60 or 50 of SEQ ID NO: 12 and/or the sequence of amino acids 1 to about 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 70, 60 or 50 of SEQ ID NO: 14. In a particular embodiment, the Aβ42-selective antibody used in assessing the Aβ42 levels of samples is antibody A387 provided herein. In another embodiment of the methods for identifying or screening for an agent that selectively modulates the level of an Aβ peptide, the Aβ39 levels of samples are assessed by contacting a sample with an antibody (or portion or fragment thereof) that selectively binds to Aβ39 (*e.g.*, the sequence of amino acids 1-39 of SEQ ID NO: 4) or by mass spectrometric analysis of the samples. Antibodies selective for Aβ39 can be prepared using methods described herein. In particular methods, an agent that modulates Aβ42 levels or Aβ39 levels or that modulates both Aβ42 and Aβ39 levels is identified. In a particular method, an agent that reduces Aβ42 levels and/or increases Aβ39 levels is identified.

The process of further identifying an agent that alters the level of one or more other Aβ peptides to a lesser extent than it alters a particular Aβ peptide or that does not substantially alter the level of the one or more other Aβ peptides can also be carried out in a number of ways. In general, this process can involve a comparison of the level of one or more other Aβ peptides in a sample that has been contacted with the agent (test sample) with that of a sample that has not been contacted with the agent (control sample). If the difference in the levels of the one or more other Aβ peptides in the test and control samples is less than the difference in the levels of the particular modulated Aβ peptide in test and control samples, or if the levels of the one or more other Aβ peptides in the test and control samples do not differ substantially (or are substantially unchanged), then the agent is identified as one that selectively modulates the level of an Aβ peptide. The skilled artisancan select appropriate concentrations of test agents at which to make such comparisons. For example, the comparison can be made at or near the EC_{50} or IC_{50} concentration for the modulation of the target Aβ peptide. If the method is for identifying an agent that selectively modulates the level of an Aβ peptide relative to only one other Aβ peptide, then the process of assessing the extent to which an agent may alter the levels of the one other Aβ peptide can involve an assessment of the levels of the

one other peptide in test and control samples using an antibody selective for the one other peptide. If the method is for identifying an agent that selectively modulates the level of an Aβ peptide relative to most or all other Aβ peptides, then the process of assessing the extent to which an agent can alter the levels of most or all other Aβ peptides can involve an assessment of the levels of all Aβ peptides in test and control samples using an antibody that recognizes most or all forms of Aβ. If the ratio of the level of the modulated Aβ peptide to the level of all Aβ peptides differs in the control and test samples, then the agent is identified as one that selectively modulates the level of the modulated Aβ peptide relative to most or all other Aβ peptides. In a particular embodiment of such a method, the antibody that recognizes most or all forms of Aβ in a sample is one that binds to Aβ1-12 (*e.g.*, the sequence of amino acids 1-12 of SEQ ID NO: 4). In one embodiment, the antibody is one that is provided herein, such as an antibody that contains the sequence of amino acids 1 to about 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 70, 60 or 50 of SEQ ID NO: 16 and/or the sequence of amino acids 1 to about 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 70, 60 or 50 of SEQ ID NO: 18. In a particular embodiment, the Aβ1-12 antibody used in assessing the Aβ peptide levels of samples is antibody B436 provided herein.

In method of identifying an agent that selectively modulates the level of an Aβ peptide relative to one or more other Aβ peptides, the identification of the Aβ-modulating agent and the determination as to what extent, if any, the agent alters the level of one or more other Aβ peptides can be conducted sequentially or simultaneously. For example, an agent that modulates the levels of an Aβ peptide can be identified by a difference in the levels of the Aβ peptide in samples contacted with the agent (test sample) and samples not contacted with the agent (control samples). That agent can then be separately evaluated for its effects on the levels of one or more other Aβ peptides by comparing the levels of the one or more other Aβ peptides in samples contacted with the agent and not contacted with the agent. Alternatively, the levels of the particular Aβ peptide to be modulated and the levels of the one or more other Aβ peptides in a test sample can be assessed and compared to the levels of the particular Aβ peptide to be modulated and the levels of the one or more other Aβ peptides in a control sample

simultaneously to, in one step, identify an agent that selectively modulates the level of an Aβ peptide.

In another method for identifying an agent that selectively modulates the level of an Aβ peptide relative to one or more other Aβ peptides, the test agent is one that is already known to modulate the level of one or more particular Aβ peptides. Thus, in one embodiment of this method, a sample containing APP or portion(s) thereof is contacted with a test agent that modulates the level of an Aβ peptide, and a test agent is identified as an agent that selectively modulates Aβ levels if the test agent does not substantially alter the level of one or more Aβ peptides other than the Aβ peptide that is modulated by the test agent. As described herein, the agent that modulates the level of an Aβ peptide that is used in this method can be one that was identified by a process involving contacting a sample containing APP or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more Aβ peptides, the processing of APP and/or the level of one or more Aβ peptides.

An agent that selectively modulates the levels of an Aβ peptide relative to one or more other Aβ peptides can alter the levels of the selectively modulated Aβ peptide(s) to a greater extent than it alters the levels of the one or more other Aβ peptides (i.e., the peptides that are not targeted for modulation). The extent to which the agent alters the levels of the particular Aβ peptide is generally significantly greater than the extent to which the agent alters the levels of one or more other Aβ peptides; that is, the greater extent of modulation is reproducible and not merely within the level of experimental error or variation. The modulation of a particular Aβ peptide by the agent can be identified by a detectable difference in the levels of the Aβ peptide in samples contacted with the agent (test sample) and samples not contacted with the agent (control samples). The agent is one that selectively modulates the levels of the particular Aβ peptide if any difference (including, for example, absolute and/or percentage difference) in the levels of one or more other Aβ peptides in samples contacted with the agent and samples not contacted with the agent is less than the difference (including, for example, absolute and/or percentage difference) in the levels of the particular Aβ peptide in test and control samples. In particular embodiments, the extent to which the agent alters the levels of one

or more other Aβ peptides (i.e., the peptides that are not targeted for modulation) is less than about 40%, 35%, 30%, 25%, or 20%. In one embodiment, the extent to which the agent alters the levels of one or more other Aβ peptides (i.e., the peptides that are not targeted for modulation) is less than 20%.

In a particular embodiment of the methods of identifying an agent that selectively modulates the level of an Aβ peptide, an agent is identified that modulates the level of an Aβ peptide without substantially altering the levels of one or more other Aβ peptides. Any modulation of the level of the one or more other Aβ peptides (i.e., the peptides that are not targeted for modulation) that is not a substantial alteration is one that is generally not associated with any significant undesired or adverse consequence in a biological context, such as, for example, in a cell, cell medium, tissue or organism.

(2) Assessment of presenilin substrate selectivity

An agent that modulates Aβ levels may act by modulating any one or more of the numerous mechanisms and activities, and components thereof, involved in Aβ synthesis through APP processing and in Aβ catabolism, degradation and clearance. One activity involved in the generation of Aβ is the presenilin/γ-secretase that participates in the processing and cleavage of APP. Any non-specific modulation of this activity could possibly effect other mechanisms in addition to APP cleavage due to the multiplicity of substrates and mechanisms with which presenilin and γ-secretase are involved. Such non-specific actions of an Aβ-modulating agent could result in undesired and adverse side effects of the modulation process.

Agents that more specifically or selectively modulate Aβ levels can be identified using methods provided herein that involve identifying agents that modulate Aβ levels without substantially altering or affecting non-APP substrate cleaving/processing activity of presenilin. These methods can involve the methods of assessing presenilin and/or presenilin-dependent activity provided and described herein.

One method provided herein for identifying or screening for agents that selectively modulate Aβ levels includes steps of contacting a sample containing a presenilin substrate, and/or portion(s) thereof, other than APP with a test agent that modulates Aβ levels and identifying a test agent as an agent that selectively modulates

$A\beta$ levels if the agent does not substantially alter the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of the presenilin substrate, and/or portion(s) thereof, that is other than APP. The sample used in this method can contain presenilin. The agent that modulates $A\beta$ levels that is used in this method can be any agent known to modulate $A\beta$ levels. The agent can, for example, be one that is identified by a method described herein which involves contacting a sample containing APP and/or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more $A\beta$ peptides, the processing of APP and/or the level of one or more $A\beta$ peptides.

The step of identifying an agent that does not substantially alter the cleavage and/or processing of the presenilin substrate, or portion(s) thereof, that is other than APP can be carried out in a number of ways. In general, this process can involve a comparison of the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of a presenilin substrate (and/or portion(s) thereof) other than APP, and/or the levels of a peptide fragment(s) of the presenilin substrate, in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). If the cleavage and/or processing of the presenilin substrate that is other than APP, and/or the substrate fragment(s) levels, of the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more $A\beta$ peptides without substantially altering the cleavage of the presenilin substrate, or portion(s) thereof, that is other than APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In a particular embodiment, the cleavage and/or processing of the presenilin substrate that is other than APP, and/or the substrate fragment(s) levels, of the test sample can be compared to that of a positive control sample. A positive control sample can be a sample that has been contacted with a known modulator of presenilin or presenilin-dependent activity. In one example, the known modulator is an inhibitor of presenilin or presenilin-dependent activity. A particular example is DAPT, which is an

inhibitor of presenilin-dependent γ-secretase activity. When a positive control is used, an agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the cleavage of the presenilin substrate, if the cleavage and/or processing of the presenilin substrate and/or the substrate fragment(s) levels of the test sample differ significantly and/or substantially from that of the positive control sample. For example, a substrate fragment(s) level of the test sample can differ from that of the positive control such that the test sample levels are less than about 40%, 35%, 30%, or 20% of the positive control sample levels. In a particular embodiment the test sample levels can be less than or equal to about 20% of the control sample levels. In one such embodiment, the positive control sample is one that has been contacted with DAPT (with a presenilin substrate fragment level set as 100%) and the test sample levels of the fragment are less than or equal to about 20% of the positive control sample levels.

In a particular embodiment, the agent that modulates Aβ levels that is used in the method is one that modulates the levels of Aβ42. In a further embodiment, the agent can be one that selectively modulates the levels of Aβ42 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In a particular embodiment, the agent modulates the levels of Aβ42 and Aβ39 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In one embodiment, the agent reduces Aβ42 levels and/or increases Aβ39 levels. Thus, using the agent identification and screening methods provided herein in combination, it is possible to identify agents that reduce Aβ42 levels without substantially altering the levels of Aβ40 or the non-APP substrate cleavage/processing activity of presenilin (i.e., with an inhibitory profile (Aβ42(+), Aβ40(-), presenilin (-))).

Another method provided herein for identifying or screening for an agent that selectively modulates Aβ levels includes steps of contacting a sample containing APP and/or a portion(s) thereof (including, for example one or more Aβ peptides) and a presenilin substrate, and/or portion(s) thereof, that is other than APP with a test agent and identifying an agent that alters the Aβ peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of Aβ and/or the levels of one or more Aβ peptides without substantially altering the cleavage (in particular, the presenilin-

dependent cleavage) of the presenilin substrate, or portion thereof, that is not APP. The sample used in this method can contain presenilin. The process of identifying an agent that alters the cleavage of APP that produces one or more Aβ peptides, the processing of APP or Aβ, and/or the level of one or more Aβ peptides can be carried out in a number of ways as described herein.

The process of further identifying an agent that does not substantially alter the cleavage of a presenilin substrate (other than APP), or portion(s) thereof, can also be carried out in a number of ways, as described herein. In general, this process can involve a comparison of the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of a presenilin substrate (or portion(s) thereof) other than APP, and/or the levels of a peptide fragment(s) of the presenilin substrate, in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). If the cleavage and/or processing of the presenilin substrate that is other than APP and/or the substrate fragment(s) levels of the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of the presenilin substrate, or portion(s) thereof, that is other than APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In a particular embodiment of these methods, the cleavage and/or processing of the presenilin substrate that is other than APP, and/or the substrate fragment(s) levels, of the test sample can be compared to that of a positive control sample. A positive control sample can be a sample that has been contacted with a known modulator of presenilin or presenilin-dependent activity. In one example, the known modulator is an inhibitor of presenilin or presenilin-dependent activity. A particular example is DAPT, which is an inhibitor of presenilin-dependent γ-secretase activity. When a positive control is used, an agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the cleavage of the presenilin substrate, if the cleavage and/or processing of the presenilin substrate and/or the substrate fragment(s) levels of the test

sample differ significantly and/or substantially from that of the positive control sample. For example, a substrate fragment(s) level of the test sample can differ from that of the positive control such that the test sample levels are less than about 40%, 35%, 30%, or 20% of the positive control sample levels. In a particular embodiment the test sample levels can be less than or equal to about 20% of the control sample levels. In one such embodiment, the positive control sample is one that has been contacted with DAPT (with a presenilin substrate fragment level set as 100%) and the test sample levels of the fragment are less than or equal to about 20% of the positive control sample levels.

In one method of identifying an agent that selectively modulates Aβ levels, the identification of the Aβ-modulating agent and the determination as to whether the agent alters the cleavage and/or processing of a presenilin substrate (other than APP), and/or portion(s) thereof, can be conducted sequentially or simultaneously. For example, when conducting the processes sequentially, an agent that modulates Aβ levels can be identified by a difference in the Aβ-producing cleavage of APP, the processing of APP or Aβ, and/or the level of one or more Aβ peptides in samples contacted with the agent (test sample) and samples not contacted with the agent (control sample). The identified agent can then be separately evaluated for its effect on presenilin substrate cleavage by comparing the cleavage and/or processing (in particular, presenilin-dependent cleavage and/or processing) of the presenilin substrate and/or the levels of a peptide fragment or fragments of the presenilin substrate in samples contacted with the test agent and not contacted with the test agent. In this sequential method, the sample used in the identification of the Aβ-modulating agent can be of the same or different type relative to the sample used in the determination as to whether the agent alters the cleavage of a presenilin substrate. If the same type of sample is used, it can contain APP (and/or portion(s) thereof) and a presenilin substrate (and/or portion(s) thereof) other than APP. The sample can also contain presenilin. If different types of samples are used, the sample used in the identification of the Aβ-modulating agent can contain APP and/or portion(s) thereof, and the sample used in the determination of alteration in the cleavage of the presenilin substrate can contain a presenilin substrate (and/or portion(s) thereof) other than APP. The sample may also contain presenilin.

Alternatively, in a simultaneously performed method, a test sample containing APP (and/or portion(s) thereof) and a presenilin substrate (and/or portion(s) thereof) other than APP can be contacted with a test agent, and the Aβ-producing cleavage of APP, the processing of APP and/or Aβ, and/or the level of one or more Aβ peptides can be assessed for the test sample, as can presenilin substrate cleavage be assessed for the same test sample. The sample may also contain presenilin. The Aβ peptide-producing cleavage or processing of APP, processing of Aβ and/or levels of Aβ peptides in the test sample, as well as the presenilin substrate cleavage of the test sample, can be compared to that of a control sample in one step to identify an agent that modulates Aβ levels without substantially altering the cleavage of a presenilin substrate (or portion(s) thereof). In particular embodiments of any of the methods, a step in the method can be identifying an agent that modulates Aβ42 levels without substantially altering the cleavage and/or processing of a presenilin substrate that is other than APP. The step can include identifying an agent that modulates Aβ42 levels relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In one embodiment, the step can include identifying an agent that reduces Aβ42 levels and/or increases Aβ39 levels.

With respect to any of the methods for identifying agents that modulate Aβ levels without substantially altering or affecting the cleavage and/or processing of a presenilin substrate that is other than APP, the identified agents either do not alter the cleavage and/or processing (in particular the presenilin-dependent cleavage and/or processing) of a presenilin substrate, or alter it in a way that it is substantially unchanged. Such alterations can be determined in a number of ways. For example, an alteration of the cleavage and/or processing of the presenilin substrate that is not substantial can be one that generally is not associated with any significant undesired or adverse consequence in a biological context, such as, for example, in a cell, cell medium, tissue or organism. An alteration of the cleavage and/or processing of the presenilin substrate that is not substantial can also be one that is assessed as a difference in the processing and/or cleavage of the substrate, or the levels of a fragment(s) of the presenilin substrate, in test and control samples that is less than about 40%, 35%, 30%, 25% or 20%. In a particular embodiment of the methods, an alteration that is not substantial can be one that is

assessed as a difference in the processing and/or cleavage of the substrate, or the levels of a fragment(s) of the presenilin substrate, in test and control samples that is less than or equal to about 20%.

In the methods for identifying agents that modulate Aβ levels without substantially altering or affecting the cleavage and/or processing of a presenilin substrate that is other than APP, the presenilin substrate can be, for example, a peptide, polypeptide, protein or fragment(s) thereof that is altered (*e.g.*, proteolytically processed, at least in part) in a presenilin-dependent manner. Thus, for example, in the case of a presenilin substrate that is altered by proteolytic processing of the substrate, if presenilin is absent, or presenilin activity is inhibited or reduced, the proteolytic processing of the presenilin substrate is altered, for example by an alteration in the levels and/or composition of fragments generated from the substrate, relative to the proteolytic processing of the substrate that occurs in the presence of normal (*e.g.*, wild-type) presenilin activity. Exemplary presenilin substrates include, but are not limited to LRP, Notch, TrkB, APLP2, hIre1α, E-cadherin and Erb-B4.

Thus, in particular embodiments of the methods for identifying agents that modulate Aβ levels without substantially altering or affecting the cleavage and/or processing of a presenilin substrate that is other than APP, agents are identified that modulate the levels of one or more Aβ peptides, such as Aβ42, without substantially altering or affecting the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of Notch, LRP, E-cadherin, Erb-B4, TrkB, APLP2 and/or hIre1α. Such methods can involve, for example, comparing the levels in test and control samples of Notch nuclear intracellular carboxyl domain (NICD), LRP carboxy terminal fragments (CTFs), E-cadherin intracellular carboxyl domain (ICD), and/or Erb-B4 intracellular carboxyl domain (ICD). In a particular embodiment of the method, the levels of one or more LRP fragments, *e.g.*, LRP-CTFs, in test and control samples are compared. The processing and processing patterns of these presenilin substrates, and characteristic fragments that can be generated therefrom, are described herein.

In a particular embodiment of the methods provided herein for identifying an

agent that modulates Aβ levels without substantially altering the processing and/or cleavage of LRP, the process identifying an agent that does not substantially alter the cleavage of LRP can involve a comparison of the cleavage and/or processing of LRP, and/or the levels of a peptide fragment(s) of LRP, in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). The processing or cleavage of an LRP or fragment(s) thereof can be assessed by determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP using, for example, materials and methods described herein. Thus, the LRP composition can be evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage of LRP or altered presenilin-dependent cleavage of LRP are present and/or the level of any such fragments. Such fragments and compositions are described herein.

In one embodiment, the processing or cleavage of an LRP or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that is cleaved in the presence of a presenilin-dependent activity (presenilin-dependent γ-secretase activity), and thus absent (or present at low levels) in the presence of the presenilin-dependent activity, but that can be detected intact when the presenilin-dependent activity is altered (such that it is eliminated or reduced). In one embodiment, the presence or absence and/or level of an LRP fragment having a molecular weight of between about 25 kD and 15 kD, and, in particular, about 20 kD, is assessed. Typically, the ~ 20 kD fragment is one that is present when an LRP is not cleaved by a presenilin-dependent activity, such as one that occurs in the presence of an inhibitor of a presenilin-dependent activity such as DAPT. In a particular embodiment, the fragment is from a C-terminal portion of LRP, i.e., a CTF. The LRP fragment can be one that contains an amino acid sequence located within the sequence of amino acids 4420-4544 of SEQ ID NO:10. In a further embodiment, the fragment is one that is recognized by an antibody generated against C-terminal amino acids (*e.g.*, the C-terminal 13 amino acids) of LRP, such as, for example, the polyclonal antibody R9377 described herein.

In a particular embodiment of the methods provided herein for identifying agents that modulate Aβ levels without substantially altering or affecting the cleavage and/or

processing of a presenilin substrate that is other than APP, agents that have been identified as agents that reduce Aβ42 levels (*e.g.*, by ≥50% at, *e.g.*, 30 μM; see, for example, EXAMPLE 6) are tested for any effects on presenilin-dependent substrate processing activity by assessing the cleavage and/or processing of LRP in the presence of the Aβ42-reducing agent (test sample) and comparing it to negative and positive control samples. In this particular embodiment, LRP processing is assessed by determining the presence or absence, and, if present, the level of an ~20 kDa fragment from a C-terminal portion of LRP. The fragment can be detected, for example, using an antibody generated against the C-terminal 13 amino acids of LRP. An Aβ42-reducing agent is selected as one that does not substantially alter the cleavage and/or processing of LRP if the level of the ~20 kDa fragment of LRP in a sample that had been contacted with the agent (*e.g.*, at 30 μM) is less than about 20% of that in a positive control sample in which presenilin-dependent γ-secretase activity has been inhibited (*e.g.*, using DAPT at ~1 μM or 1 mM).

In other embodiments of the methods for identifying or screening for agents that modulate Aβ levels without substantially altering or affecting the cleavage and/or processing of a presenilin substrate that is other than APP, agents are identified that modulate the levels of one or more Aβ peptides, such as Aβ42, without substantially altering or affecting the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of Notch, E-cadherin, Erb-B4, TrkB, APLP2 and/or hIre1α. In particular embodiments, these methods can involve, for example, comparing the levels (and/or presence or absence) in test and control samples of one or more fragments of Notch, E-cadherin and/or Erb-B4 (as well as LRP) or portion(s) thereof. The methods that involved assessing processing of Notch, E-cadherin or Erb-B4 can be conducted, for example, in a manner similar to that described herein for methods that involve assessing LRP processing. Because alteration, such as, for example, inhibition, of the presenilin-dependent cleavage of Notch can result in adverse side affects including, for example, immunodeficiency and anaemia, one embodiment of the methods described herein includes screening for Aβ-modulating agents that do not substantially alter Notch cleavage and/or processing (in particular, presenilin-dependent processing). Furthermore, non-specific modulation of presenilin and/or presenilin-

dependent activity may affect E-cadherin and/or Erb-B4 processing resulting in adverse side affects and, therefore, in particular embodiments of the methods described herein, agents are identified that modulate Aβ levels without substantially altering or affecting E-cadherin and/or Erb-B4 processing. In particular embodiments, the method can involve identifying agents that modulate Aβ levels without substantially altering the cleavage and/or processing of one or more or all of LRP, Notch, E-cadherin and Erb-B4.

(3) Assessment of carboxy-terminal fragments of APP and APP AICD

In addition, other parameters of APP processing may be monitored to determine if the cellular pathway is being altered by an Aβ modulating agent in a way that may result in adverse side effects. For example, an agent that inhibits γ-secretase may cause the accumulation of high amounts of the carboxy terminal fragment species of APP cleaved by α- or β-secretase. Such fragments may be neurotoxic at high levels. Accumulation of these fragments or the N-terminal fragments produced by α- or β-secretase can be determined by immunoassaying cell lysates with an appropriate antibody prepared to such peptides.

One method provided herein for identifying or screening for agents that selectively modulate Aβ levels includes steps of contacting a sample containing APP, or portion(s) thereof and α- and/or β-secretase activity with a test agent that modulates Aβ levels and identifying a test agent as an agent that selectively modulates Aβ levels if the agent does not substantially alter the level or composition of fragments produced by α- or β-secretase. The agent that modulates Aβ levels that is used in this method can be any agent known to modulate Aβ levels. The agent can, for example, be one that is identified by a method described herein which involves contacting a sample containing APP or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more Aβ peptides, the processing of APP and/or the level of one or more Aβ peptides.

The step of identifying an agent that does not substantially alter the level or composition of fragments produced by α- or β-secretase can be carried out in a number of ways. In general, this process can involve a comparison of the α- and/or β-secretase

cleavage of APP (or portion thereof) that has been contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the level or composition of fragments produced by α- and/or β-secretase cleavage of APP (or portion thereof) in the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the α- and/or β-secretase cleavage of APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In a particular embodiment, the agent that modulates Aβ levels that is used in the method is one that modulates the levels of Aβ42. In a further embodiment, the agent can be one that selectively modulates the levels of Aβ42 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In a particular embodiment, the agent modulates the levels of Aβ42 and Aβ39 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In one embodiment, the agent reduces Aβ42 levels and/or increases Aβ39 levels.

Another method provided herein for identifying or screening for an agent that selectively modulates Aβ levels includes steps of contacting a sample containing APP and/or a portion(s) thereof (including, for example one or more Aβ peptides) and α- and/or β-secretase activity with a test agent and identifying an agent that alters the Aβ peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of Aβ and/or the levels of one or more Aβ peptides without substantially altering the α- and/or β-secretase cleavage of APP. The process of identifying an agent that selectively modulates one or more Aβ peptides can be carried out in a number of ways as described herein.

The process of further identifying an agent that does not substantially alter the level or composition of fragments produced by α- or β-secretase cleavage of APP, can also be carried out in a number of ways, as described herein. In general, this process can involve a comparison of the α- and/or β-secretase cleavage of APP (or portion thereof) that has been contacted with the test agent (i.e., test sample) and of a sample that has not

been contacted with the test agent (i.e., control sample). If the level or composition of fragments produced by α- and/or β-secretase cleavage of APP (or portion thereof) in the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the α- and/or β-secretase cleavage of APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In this method of identifying an agent that selectively modulates Aβ levels, the identification of the Aβ-modulating agent and the determination as to whether the agent alters the levels and/or composition of α- and/or β-secretase cleavage of APP, or portion thereof, can be conducted sequentially or simultaneously. For example, an agent that modulates Aβ levels can be identified by a difference in the processing of APP or Aβ, and/or the level of one or more Aβ peptides in samples contacted with the agent (test sample) and samples not contacted with the agent (control sample). The identified agent can then be separately evaluated for its effects on α- and/or β-secretase cleavage of APP by comparing the α- and/or β-secretase cleavage of APP (or portion thereof) in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). In this sequential method, the sample used in the identification of the Aβ-modulating agent can be of the same or different type relative to the sample used in the determination as to whether the agent alters the α- and/or β secretase cleavage of APP. If the same sample is used, it can contain APP (and/or portion(s) thereof) and an α- and/or β-secretase. If different types of samples are used, the sample used in the identification of the Aβ-modulating agent can contain APP and/or portion(s) thereof, and the sample used in the determination of alteration in the α- and/or β-secretase cleavage of APP can contain APP and/or portion(s) thereof, and an α- and/or β-secretase.

Alternatively, in a simultaneously performed method, a test sample containing APP (and/or portion(s) thereof), and an α- and/or β-secretase can be contacted with a test agent and the cleavage of APP that produces one or more Aβ peptides, the processing of APP or Aβ, and/or the level of one or more Aβ peptides can be assessed for the test

sample, as can α- and/or β-secretase cleavage of APP be assessed for the same test sample. The Aβ peptide-producing cleavage or processing of APP, processing of Aβ and/or levels of Aβ peptides of the test sample, as well as the α- and/or β-secretase cleavage of APP of the test sample, can be compared to that of a control sample in one step to identify an agent that modulates Aβ levels without substantially altering the level or composition of fragments produced by α- and/or β-secretase cleavage of APP (or portion thereof).

In the embodiments of the methods provided herein for identifying agents that modulate Aβ levels without substantially altering or affecting the level or composition of fragments produced by α- and/or β-secretase cleavage of APP (or portion thereof), the fragments produced by α- and/or β-secretase cleavage of APP (or portion thereof) can be detected by any methods known in the art or described herein, for example, using an antibody generated against the amino acids of sAPPα, C83, p3, sAPPβ, or C99.

Further studies have demonstrated the production of an intracellular CTF of APP resulting from γ-secretase cleavage, which, in analogy to NICD, is referred to as AICD (APP intracellular domain) (Pinnix, I *et al.* (2001) *J. Biol. Chem 276*:481-487; Sastre, M. *et al.* (2001) *EMBO Reports 2(9)*:835-41; Gu, Y *et al.* (2001) *J. Biol Chem. 276(38)*: 35235-8). Sequencing has revealed that its N-terminus does not correspond to the expected γ-secretase cleavage after amino acids 40 or 42 of the Aβ domain. Instead, cleavage occurs between amino acids 49 and 50, close to the cytoplasmic side of the transmembrane domain. Amino acids 49 and 50 of the Aβ domain correspond to amino acids 720 and 721 of the full length APP protein (see e.g., amino acids 720 and 721 of SEQ ID NOs. 2 and 28). This cleavage is reminiscent of the S3 cleavage of Notch and may thus indicate an analogous function of AICD in signal transduction. Indeed, the cytoplasmic fragment of APP has been shown to form a transcriptionally active complex with Fe65, and Tip60 (Cao, X and Sudhof, T.C. (2001) *Science 293*:115-120). Inhibition of such cleavage may result in unwanted side affects. Thus, in a particular embodiment, a fragment of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain (close to the cytoplasmic side of the transmembrane domain) is substantially unchanged in the presence of a test agent when compared to that in the absence of the test

agent.

One method provided herein for identifying or screening for agents that selectively modulate Aβ levels includes steps of contacting a sample containing APP, or portion(s) thereof and γ-secretase activity with a test agent that modulates Aβ levels and identifying a test agent as an agent that selectively modulates Aβ levels if the agent does not substantially alter the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain. The agent that modulates Aβ levels that is used in this method can be any agent known to modulate Aβ levels. The agent can, for example, be one that is identified by a method described herein which involves contacting a sample containing APP or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more Aβ peptides, the processing of APP and/or the level of one or more Aβ peptides.

The step of identifying an agent that does not substantially alter the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain can be carried out in a number of ways. In general, this process can involve a comparison of the γ-secretase cleavage of APP (or portion thereof) that has been contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the level or composition of fragments produced by γ-secretase cleavage of APP (or portion thereof) with an N-terminal end that terminates after amino acid 49 of the Aβ domain in the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the level or composition of fragments of APP with an N-terminal end that terminates after amino acid 49 of the Aβ domain. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In a particular embodiment, the agent that modulates Aβ levels that is used in the method is one that modulates the levels of Aβ42. In a further embodiment, the agent can be one that selectively modulates the levels of Aβ42 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In a particular embodiment, the agent

modulates the levels of Aβ42 and Aβ39 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In one embodiment, the agent reduces Aβ42 levels and/or increases Aβ39 levels.

Another method provided herein for identifying or screening for an agent that selectively modulates Aβ levels includes steps of contacting a sample containing APP and/or a portion(s) thereof (including, for example one or more Aβ peptides) and γ-secretase activity with a test agent and identifying an agent that alters the Aβ peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of Aβ and/or the levels of one or more Aβ peptides without substantially altering the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain. The process of identifying an agent that selectively modulates one or more Aβ peptides can be carried out in a number of ways as described herein.

The process of further identifying an agent that does not substantially alter the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain, can also be carried out in a number of ways, as described herein. In general, this process can involve a comparison of the γ-secretase cleavage of APP (or portion thereof) that has been contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain in the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In this method of identifying an agent that selectively modulates Aβ levels, the identification of the Aβ-modulating agent and the determination as to whether the agent alters the levels and/or composition of fragments of APP having an N-terminal end that

terminates after amino acid 49 of the Aβ domain, or portion thereof, can be conducted sequentially or simultaneously. For example, an agent that modulates Aβ levels can be identified by a difference in the processing of APP or Aβ, and/or the level of one or more Aβ peptides in samples contacted with the agent (test sample) and samples not contacted with the agent (control sample). The identified agent can then be separately evaluated for its effects on fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain by comparing the fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain (or portion thereof) in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). In this sequential method, the sample used in the identification of the Aβ-modulating agent can be of the same or different type relative to the sample used in the determination as to whether the agent alters the level and/or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain. If the same sample is used, it can contain APP (and/or portion(s) thereof) and an γ-secretase. If different types of samples are used, the sample used in the identification of the Aβ-modulating agent can contain APP and/or portion(s) thereof, and the sample used in the determination of alteration in the fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain can contain APP and/or portion(s) thereof, and a γ-secretase activity.

Alternatively, in a simultaneously performed method, a test sample containing APP (and/or portion(s) thereof), and a γ-secretase can be contacted with a test agent and the cleavage of APP that produces one or more Aβ peptides, the processing of APP or Aβ, and/or the level of one or more Aβ peptides can be assessed for the test sample, as can fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain be assessed for the same test sample. The Aβ peptide-producing cleavage or processing of APP, processing of Aβ and/or levels of Aβ peptides of the test sample, as well as the fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain, can be compared to that of a control sample in one step to identify an agent that modulates Aβ levels without substantially altering the level or composition of fragments of APP having an N-terminal end that terminates after amino

acid 49 of the Aβ domain (or portion thereof).

In the embodiments of the methods provided herein for identifying agents that modulate Aβ levels without substantially altering or affecting the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the Aβ domain, the APP fragments having an N-terminal end that terminates after amino acid 49 of the Aβ domain can be detected by any methods known in the art or described herein, for example, using an antibody generated against the C-terminal amino acids of APP. The C-terminal amino acids may include any amino acid C-terminal to amino acid 49 of the Aβ domain or any amino acid C-terminal to amino acid 720 of full length APP.

E. Systems

There are a number of kits, combinations and systems that can be used in performing the various methods provided herein. Such methods include methods for assessing presenilin activity, methods for identifying candidate agents for treatment or prophylaxis of a disease or disorder associated with an altered presenilin, methods for identifying or screening for agents that modulate Aβ levels and methods for identifying or screening for agents for treatment or prophylaxis of a disease or disorder characterized by and/or associated with altered Aβ levels and/or processing of APP, including for example, diseases associated with amyloidosis.

Kits, combinations and systems are also provided herein. Such kits, combinations and/or systems can include, for example, a cell(s) (and/or lysates, extracts, medium and membranes from the cell(s)) exhibiting APP (altered and/or wild-type as well as portion(s) of APP) expression and processing, one or more presenilins (altered and/or wild-type as well as portion(s) of presenilins) expression and processing, and/or one or more presenilin substrates (altered and/or wild-type as well as portion(s) of presenilin substrates), including, for example, LRP, Notch, E-cadherin and Erb-B4. The cells of the system can be isolated cells or cell cultures that endogenously express such protein(s) or can recombinantly express such proteins as described above with respect to the methods for identifying agents. Systems in which the cells recombinantly express the proteins can be such that the cells are isolated cells or cell cultures or are contained within an animal, in particular, a non-human animal, *e.g.*, a non-human mammal. Many

examples of such cells are described herein and known in the art.

The kits, combinations and/or systems provided herein can include antibodies and/or fragment(s) thereof specifically reactive to particular Aβ peptides. For example, a system can include antibodies specifically reactive to Aβ42 versus one or more other Aβ peptides, and in particular, Aβ40. Aβ42 selective-antibodies are provided herein. Such antibodies can be made by the methods described herein, including, for example, by immunization with a peptidyl sequence of MVGGVVIA, and by recombinant methods. One such antibody (and/or fragment(s) thereof) includes the sequence of amino acids 1-95 of SEQ ID NO:12 and/or 1-97 of SEQ ID NO: 14. A kit, combination or system can include cells that produce any such antibody (and/or fragment(s) thereof). For example, such a cell could contain nucleic acid containing the sequence of nucleotides set forth as nucleotides 1-285 of SEQ ID NO: 11 and/or the sequence of nucleotides set forth as nucleotides 1-291 of SEQ ID NO: 13.

The kits, combinations and/or systems provided herein can include detection antibodies (and/or fragment(s) thereof) designed to be reactive to more than one species of Aβ. In one example, the antibodies that are reactive to a sequence on the N-terminus of Aβ, such as, for example amino acids 1-12 of Aβ. Such antibodies (and/or fragment(s) thereof) are provided herein and include antibodies containing one or both of the amino acids 1-100 of SEQ ID NO: 16 and 1-98 of SEQ ID NO: 18. A kit, combination or system can include cells that produce any such antibody (and/or fragment(s) thereof). For example, such a cell could contain nucleic acid containing the sequence of nucleotides set forth as nucleotides 1-300 of SEQ ID NO: 15 and/or the sequence of nucleotides set forth as nucleotides 1-294 of SEQ ID NO: 17. The detection antibody is generally conjugated to a detectable label, such as, for example alkaline phosphatase, and the presence or absence of antibody binding can be determined by luminescence of a substrate that is detected by a change in light emitted in the presence of alkaline phosphatase, such as, for example, CDP-Star chemiluminescence substrate (Tropix, Inc.).

One system provided herein can be used, for example, in assessing presenilin activity. In a particular embodiment, the system includes a source of presenilin activity, a

source of LRP (and/or portion(s) thereof) protein and a reagent for determining LRP protein composition. In one embodiment, the source of presenilin activity can be, for example, a standard or control used in a method of assessing presenilin activity. In another embodiment, the source of presenilin activity can be the activity that is being assessed. An example of a reagent for determining LRP protein composition is an antibody (and/or fragment(s) thereof) that recognizes a fragment of LRP generated by a presenilin-dependent activity, *e.g.*, presenilin-dependent γ-secretase or a LRP fragment that occurs in the absence of such activity. Such fragments include LRP-CTF, and, in particular an ~ 20 kD fragment of LRP. In one embodiment, the system includes an anti-LRP antibody prepared to the carboxyl-terminal 13 amino acid peptide of LRP (C-GRGPEDEIGDPLA). In a particular embodiment, the system includes the anti-LRP polyclonal antibody (R9377) described herein (see, *e.g.*, the EXAMPLES). Some systems can also contain sources of other presenilin substrates, *e.g.*, Notch, Erb-B4 and E-cadherin) and reagents, such as antibodies and/or fragment(s) thereof, that are reactive to Notch intracellular domain (NICD), E-cadherin intracellular domain, or Erb-B4 intracellular domain.

One embodiment of a system or kit for use in identifying agents that modulate Aβ levels provided herein contains a reagent for assessing cleavage of APP that produces one or more Aβ peptides, APP processing, Aβ processing and/or Aβ levels and a reagent for assessing cleavage and/or processing (in particular, presenilin-dependent processing) of a presenilin substrate. In a particular embodiment, the presenilin substrate is LRP and/or portion(s) thereof. Such reagents are described and provided herein. For example, reagents for assessing Aβ levels include antibodies and/or fragments thereof such as antibodies that specifically react with Aβ42, for example an antibody or fragment(s) thereof containing the sequence of amino acids 1-95 of SEQ ID NO:12 and/or 1-97 of SEQ ID NO: 14. Another example of an antibody that can be used in assessing Aβ levels is an antibody that recognizes most or all forms of Aβ. One example is an antibody (and/or fragment(s) thereof) containing one or both of the amino acids 1-100 of SEQ ID NO: 16 and 1-98 of SEQ ID NO: 18. An example of a reagent for determining LRP protein composition in assessing LRP cleavage and/or processing is an antibody (and/or

fragment(s) thereof) that recognizes a fragment of LRP generated by a presenilin-dependent activity, *e.g.*, presenilin-dependent γ-secretase or a LRP fragment that occurs in the absence of such activity. Such fragments include LRP-CTF, and, in particular an ~ 20 kD fragment of LRP. In one embodiment, the system includes an anti-LRP antibody prepared to the carboxyl-terminal 13 amino acid peptide of LRP (C-GRGPEDEIGDPLA). In a particular embodiment, the system includes the anti-LRP polyclonal antibody (R9377) described herein (see, *e.g.*, the EXAMPLES). Some systems can also contain reagents such as antibodies and/or fragment(s) thereof that are reactive to Notch intracellular domain (NICD), E-cadherin intracellular domain, or Erb-B4 intracellular domain.

F. Methods of Identifying Agents for the Treatment of a Disease or Disorder

Provided herein are methods for identifying candidate agents for the treatment or prophylaxis of diseases and disorders associated with or characterized by altered APP processing, Aβ production, catabolism, processing and/or levels. Disease models are a valuable tool for the discovery and testing of treatment agents. Such disease models may be cellular or organismal and may be produced by methods known to those of skill in the art and described herein.

1. Cell models

Cell models for the identification and testing of agents for the treatment of diseases and disorders characterized by altered Aβ peptide levels are provided herein. Suitable cell lines include human and animal cell lines, such as the 293 human kidney cell line, neuroglioma cell lines, neuroblastoma cell lines, HeLa cells, primary endothelial cells, primary fibroblasts or lymphoblasts, primary mixed brain cells (including neurons, astrocytes, and neuroglia), Chinese hamster ovary (CHO) cells, and the like.

In a particular embodiment, mixed brain cell cultures from transgenic mice (e.g., Tg2576 transgenic mice) are provided. Such primary cultures can mimic an in vivo system more closely than engineered cell lines. Primary mixed brain cultures can be established by any method known to those of skill in the art or described herein. Generally, primary mixed brain cultures can be produced by dissecting 17 day old mouse

embryos utilizing a stereo scope, obtaining brain tissue and dissociating with papain, then culturing cells by standard procedures for primary neuronal cultures.

Primary cell cultures can be obtained from any host, in a particular embodiment, a non-human host, including but not limited mice, rabbits, monkeys, apes, etc. which naturally express APP or any one or combination of isoforms or fragments of APP. The primary cultures can comprise cells that express wild type versions or isoforms of APP or mutant versions. The cells can over express the protein as well.

Alternatively, engineered cell lines may be used. Cells may contain recombinant DNA that when expressed, result in altered production, degradation or clearance of Aβ peptides or altered expression of APP, such as by replacing or modifying the promoter region or other regulatory region of the endogenous gene. Such a cell can by produced by introduction of heterologous or homologous nucleic acid into the cell using methods known in the art and described herein. In a particular embodiment, the cell is a recombinant cell that expresses the protein(s) as heterologous protein(s). Such cells may overexpress or mis-express the heterologous protein(s). For example, a recombinant cell may be one that endogenously expresses the protein(s) and also has been transfected with additional copies of nucleic acid encoding the protein(s). Alternatively, the host cell used in the generating the recombinant cell may be one that endogenously expresses little to none of the protein(s) of interest or one in which such proteins have been eliminated (*e.g.*, through gene knock-out methods or by inhibition with an agent that does not inhibit the activity of the heterologous protein(s)). In a particular embodiment, cell lines capable of expressing APP variants with altered Aβ peptide levels are provided. Such variants can include those having one or several amino acid substitutions directly amino-terminal of the Aβ cleavage site. For example, APP DNA bearing a double mutation (Lys^{595}->Asn^{595} and Met^{596}->Leu^{596}) found in a Swedish FAD family produce approximately six-to-eight fold more Aβ than cells expressing normal APP. Exemplary clones and vectors for APP include but are not limited ATCC accession numbers 40305, 40347, 78397, 78510, 78510D, 86195.

Cells or less differentiated precursor cells may be stably or transiently transfected with purified or recombinant protein(s) in vitro or in an organism. In vitro transfection is

followed by cell expansion through culturing prior to use. Cells from a known cell line are preferred, such as from neuroblastoma SH-SY5Y cells, pheochromocytoma PC12 cells, neuroblastoma SK-N-BE(2)C cells, human SK-N-MC neuroblastoma cells, SMS-KCNR cells, human LAN-5 neuroblastoma cells, human GI-CA-N neuroblastoma cells, human neuroblastoma cells, mouse Neuro 2a (N2A) neuroblastoma cells and/or human IMR 32 neuroblastoma cells. Exemplary cell lines include human embryonic kidney 293 (HEK 293) ATCC accession number CRL-1573, CHO (including CHO and CHO-K1(accession number CCL-61)), LTK$^-$, N2A (accession number CCL-131), H6, and HGB. The generation, maintenance and use of such cell lines is well known.

Suitable cells include mammalian cell lines, typically human cell lines that are commercially available for example from the American Type Tissue Culture Collection (ATCC), Rockville, Maryland, 20852. Exemplary cells include CHO cells expressing human APP751 from a vector containing the gene encoding APP751, human mutant APPP751 (V717F) from a vector containing a gene encoding APP751 (V717F), or a combination thereof and can be cultured in standard cell culture media supplemented with 10% fetal calf serum and optionally with antibiotics and fungicides such as 100 U/mL penicillin/streptomycin. Other suitable cells include human neuroglioma cells HS683 that express APP695, APP751, APP770 or a combination thereof from a vector containing a gene encoding for the respective protein or partial protein. Additionally, a human neuroblastoma cell line SH-SY5Y described in T. Yamazaki and Y. Ihara (1998) Neurobiology of Aging 19:S77-S79 or other cell that secretes large amounts of Aβ into the medium without Aβ transfection can also be used.

An exemplary transformed human embryonic kidney cell line is the human 293 cell line, ATCC accession number CRL-1573. Other suitable cells include CRL-1721 and CCL-92 and those listed in the catalogue from the Indiana Alzheimer Disease Center National Cell Repository of Indiana University - Purdue University Indianapolis, 425 University Blvd., Indianapolis, IN 46202-5143, which is incorporated by reference herein in its entirety.

Additionally, primary cell cultures, immortalized cell lines, or stem cells (embryonic or adult) induced to express Aβ proteins or peptides can be used. In one

embodiment, cells that are not terminally differentiated can be induced to express neuronal characteristics. Such cells can be induced for example by exposing them to a growth factor, cyotokine, hormone, neural inducing media or combination thereof.

2. Animal models

Animal models for the identification and testing of agents for the treatment of diseases and disorders characterized by altered Aβ peptide levels are provided herein. Transgenic animal models and animals, such as rodents, including mice and rats, cows, chickens, pigs, goats, sheep, monkeys, including gorillas, and other primates, are provided herein. In particular, transgenic non-human animals that contain recombinant DNA that when expressed, result in altered production, degradation and/or clearance of Aβ peptides or altered expression of APP, such as by replacing or modifying the promoter region or other regulatory region of the endogenous gene are provided. Such an animal can by produced by promoting recombination between endogenous nucleic acid and an exogenous gene of interest that could be over-expressed or mis-expressed, such as by expression under a strong promoter, via homologous or other recombination event.

Transgenic animals can be produced by introducing the nucleic acid using any know method of delivery, including, but not limited to, microinjection, lipofection and other modes of gene delivery into a germline cell or somatic cells, such as an embryonic stem cell. Typically the nucleic acid is introduced into a cell, such as an embryonic stem cell (ES), followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, which is followed by the birth of a transgenic animal. Generally introduction of a heterologous nucleic acid molecule into a chromosome of the animal occurs by a recombination between the heterologous nucleic acid of interest and endogenous nucleic acid. The heterologous nucleic acid can be targeted to a specific chromosome.

In some instances, knockout animals can be produced. Such an animal can be initially produced by promoting homologous recombination between an gene of interest in its chromosome and the corresponding exogenous gene of interest that has been rendered biologically inactive (typically by insertion of a heterologous sequence, *e.g.*, an antibiotic resistance gene). In one embodiment, this homologous recombination is

performed by transforming embryo-derived stem (ES) cells with a vector containing the insertionally inactivated gene of interest, such that homologous recombination occurs, followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of the chimeric animal ("knockout animal") in which a gene of interest has been inactivated (see Capecchi, *Science* 244:1288-1292 (1989)). The chimeric animal can be bred to produce homozygous knockout animals, which can then be used to produce additional knockout animals. Knockout animals include, but are not limited to, mice, hamsters, sheep, pigs, cattle, and other non-human mammals. For example, a knockout mouse is produced. The resulting animals can serve as models of specific diseases that are the result of or exhibit altered-expression of a polypeptide involved in neurodegenerative disorders. Such knockout animals can be used as animal models of such diseases *e.g.*, to screen for or test molecules for the ability to treat or prevent such diseases or disorders.

Other types of transgenic animals also can be produced, including those that over-express a polypeptide involved in neurodegenerative disorders. Such animals include "knock-in" animals that are animals in which the normal gene is replaced by a variant, such a mutant, an over-expressed form, or other form. For example, one species', such as a rodent's endogenous gene can be replaced by the gene from an other species, such as from a human. Animals also can be produced by non-homologous recombination into other sites in a chromosome; including animals that have a plurality of integration events.

After production of the first generation transgenic animal, a chimeric animal can be bred to produce additional animals with over-expressed or mis-expressed polypeptides involved in neurodegenerative disorders. Such animals include, but are not limited to, mice, hamsters, sheep, pigs, cattle and other non-human mammals. The resulting animals can serve as models of specific diseases that are the result of or exhibit over-expression or mis-expression of a polypeptide involved in neurodegenerative disorders. Such animals can be used as animal models of such diseases *e.g.*, to screen for or test molecules for the ability to treat or prevent such diseases or disorders. In a specific embodiment, a mouse with over-expressed or mis-expressed APP is produced.

One useful non-human animal model harbors a copy of an expressible transgene

sequence which encodes the Swedish mutation of APP (Asp595-leu596). US Patent Nos. 5,612,486 and 5,850,003, incorporated herein by reference, disclose a transgenic rodent having a diploid genome comprising a transgene encoding a heterologous APP polypeptide having the Swedish mutation wherein the amino acid residues at positions corresponding to positions 595 and 596 in human APP695 are asparagine and leucine, respectively. The transgene is expressed to produce a human APP polypeptide having the Swedish mutation. The polypeptide is processed in a sufficient amount to be detectable in a brain homogenate of the transgenic rodent. The sequence generally is expressed in cells which normally express the naturally-occurring endogenous APP gene (if present). Murine and hamster models are suitable for this use. Such transgenes typically comprise a Swedish mutation APP expression cassette, in which a linked promoter and, preferably, an enhancer drive expression of structural sequences encoding a heterologous APP polypeptide comprising the Swedish mutation.

Other suitable animal models include the transgenic mouse disclosed in US Patent No. 5,387,742. This transgenic mouse contains a DNA sequence with a nerve tissue specific promoter and a DNA sequence which encodes a β-amyloid precursor protein selected from the group consisting of A751 and A770. The promoter and DNA sequence which encodes the precursor protein are operatively linked to each other and integrated in the genome of the mouse and expressed to form β-amyloid protein deposits in the brain of the mouse.

Still other transgenic animal models for the identification and testing of agents for the treatment of disease and disorders characterized by altered Aβ peptide levels include those described in US Patent Nos. 5,811,633; 6,037,521; 6,184,435; 6,187,992; 6,211,428; and 6,340,783, all of which are incorporated by reference, transgenic mouse models Tg 2576; APPSWE mouse, K670N, M671L, and other models including APP(V717F), APP(K670N, M671L and V717F), PS-1 M146L, PS-1 M146V, APPSWE + PS A246E (reviewed by Emilien, *et al.*, (2000) *Arch. Neuro. 57*: 176-81).

3. Evaluation of models and identification and testing of agents for the treatment of diseases and disorders

Cell and animal models of diseases and disorders involving Aβ misregulation

described herein have a number of uses. For example, by evaluating the cellular or organismal phenotypes associated with the altered expression of proteins involved in Aβ regulation in the cells/organisms and correlating such phenotypes with specific cellular molecules and processes, the disease/disorder models can be used in elucidating the mechanisms underlying Aβ misregulation in a cell as well as in dissecting processes and pathways involved in Aβ regulation. In addition, by evaluating the effects of test agents or candidate therapeutic agents on Aβ levels and the phenotypic manifestations of the model cells/organisms, the models can be used in screening agents and testing candidate agents for the treatment of diseases and disorders that involve Aβ misregulation.

In the methods for identifying agents for the treatment or prophylaxis of a disease or disorder, any sample containing an altered test protein, and/or portion(s) thereof, and APP, and/or portion(s) thereof, wherein the altered protein is associated with altered Aβ42 production, catabolism, processing and/or Aβ42 levels may be used. Such samples can include, for example any cell, cell extract, cell model, organism or animal model described herein. The cell, organism or animal may be one that contains an altered APP, APP processing activity, or Aβ processing activity and/or expresses altered Aβ levels such as, for example, the cell and animal models described above. The altered APP, APP processing activity, Aβ processing activity, or Aβ level can be one that is altered relative to a wild-type. Typically, a wild-type protein, such as, for example, APP, APP processing enzyme or Aβ processing enzyme can be one that is encoded by a predominant allele in a population or any allele that is not associated with disease or a pathogenic condition. A wild-type APP, APP processing enzyme or Aβ processing enzyme can be one that occurs in an organism that exhibits normal APP and/or Aβ processing patterns. The altered APP, APP processing enzyme or Aβ processing enzyme can be a mutant or can be, for example, one that is encoded by a nucleic acid linked to Alzheimer's disease. For example, the altered enzyme activity may include any one or more of the at least 60 mutations in human PS1 and the at least two mutations in human PS2 that have been genetically linked to early onset familial Alzheimer's disease (FAD). Exemplary presenilins with altered activity include FAD-associated mutant forms of PS1 and PS2 that give rise to an increased accumulation of Aβ42 in AD patients and

transfected cell lines and transgenic animals in which they are expressed. Included among such mutations are the PS2 FAD mutation N141I (Volga German FAD mutant) and the PS1 FAD mutation M146L. Examples of diseases associated with an altered APP, APP processing activity, Aβ, and/or Aβ processing activity for which the methods provided herein can be used to identify candidate therapeutic or prophylactic agents include, but are not limited to, amyloidosis-associated diseases and neurodegenerative diseases. In a particular aspect, the disease is Alzheimer's Disease.

In one method for testing an agent for use in the treatment of a disease or disorder, the test agent is one that is already known to modulate the level of one or more particular Aβ peptides. Thus, in one embodiment of this method, a disease model is contacted with a test agent that modulates the level of an Aβ peptide, and a test agent is identified as an agent for the treatment of a disease or disorder if the test agent at least partially reverses or reduces, ameliorates or eliminates a disease trait or phenotype exhibited by a model cell or organism, or that tends to restore APP processing and/or Aβ processing or levels to compensate for disease-associated abnormalities in Aβ levels. In general, the step of identifying a test agent that at least partially reverses or reduces, ameliorates or eliminates a disease trait or phenotype exhibited by a model cell or organism, or that tends to restore APP processing and/or Aβ processing or levels can involve a comparison of the disease trait or phenotype and/or APP processing and/or Aβ processing or levels in a model that has been contacted with the test agent (i.e., test model) and in a model that has not been contacted with the test agent (i.e., control model). If the disease trait or phenotype and/or APP processing and/or Aβ processing or levels in the test and control models differs, then the test agent is identified as a candidate agent for the treatment and/or prophylaxis of a disease or disorder. In such an embodiment, both the test and control model express the disease trait or phenotype in the absence of the test agent. In another embodiment, the control model or sample is a wild type model or sample. In such an embodiment, the step of identifying a candidate agent includes comparing the disease trait or phenotype and/or Aβ production, catabolism, processing and/or Aβ levels in a test sample that has been contacted with the test agent and a positive control sample and identifying an agent as a candidate agent Aβ

production, catabolism, processing and/or Aβ levels if Aβ production, catabolism, processing and/or Aβ levels in the test and control samples is substantially similar

The agent that modulates the level of an Aβ peptide that is used in this method can be one that was identified by any of the processes described herein. For example, the agent may be one that was identified by a process involving contacting a sample containing APP or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more Aβ peptides, the processing of APP and/or the level of one or more Aβ peptides.

The agent that modulates the level of an Aβ peptide that is used in this method can be one that was identified as having a particular selectivity. Methods of assessing the selectivity of an Aβ modulating agent are provided herein. In a particular embodiment the agent that selectively modulates Aβ levels can be one that does not substantially alter the level of one or more Aβ peptides other than the Aβ peptide that is modulated by the test agent. In a particular embodiment, the agent that modulates Aβ levels that is used in the method is one that modulates the levels of Aβ42. In a further embodiment, the agent can be one that selectively modulates the levels of Aβ42 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In a particular embodiment, the agent modulates the levels of Aβ42 and Aβ39 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In one embodiment, the agent reduces Aβ42 levels and/or increases Aβ39 levels. In a particular embodiment, the agent that reduces Aβ42 levels does not substantially alter the levels of non-APP substrate cleavage/processing activity of presenilin, such as LRP and/or other substrates provided herein. In anther embodiment the agent that reduces Aβ42 levels does not substantially alter the levels of Aβ40 or the non-APP substrate cleavage/processing activity of presenilin.

In other embodiments, agents that have not previously been screen for their ability to modulate the level of one or more particular Aβ peptides may be screened in cellular and organismal disease model systems. An agent can be identified as an agent that alters the cleavage of APP that produces one or more Aβ peptides, the processing of APP or Aβ, and/or the level of one or more Aβ peptides. In one embodiment, an alteration results in the restoration of APP processing and/or Aβ processing or levels to

compensate for disease-associated abnormalities in Aβ levels. At the same time, the agent can be identified as an agent that at least partially reverses or reduces, ameliorates or eliminates a disease trait or phenotype exhibited by a model cell or organism. The process of identifying an alteration in APP processing, Aβ processing and Aβ levels can be carried out in a number of ways as described herein.

The selectivity of the agent may also be assessed in the disease model system. Any methods of assessing the selectivity of an Aβ modulating agent provided herein may be used. In a particular embodiment the agent that selectively modulates Aβ levels does not substantially alter the level of one or more Aβ peptides other than the Aβ peptide that is modulated by the test agent. In a particular embodiment, the agent that modulates Aβ levels that is used in the method is one that modulates the levels of Aβ42. In a further embodiment, the agent can be one that selectively modulates the levels of Aβ42 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In a particular embodiment, the agent modulates the levels of Aβ42 and Aβ39 relative to Aβ40 levels and/or the levels of all or most of the other forms of Aβ. In one embodiment, the agent reduces Aβ42 levels and/or increases Aβ39 levels. The modulation of a particular Aβ peptide by the agent can be identified by any of the methods described herein. In general, the modulation of a particular Aβ peptide by the agent can be identified by a detectable difference in the levels of the Aβ peptide in the model cell or organism contacted with the agent (test model) and model cells or organisms not contacted with the agent (control models). The agent is one that selectively modulates the levels of the particular Aβ peptide if any difference (including, for example, absolute and/or percentage difference) in the levels of one or more other Aβ peptides in model contacted with the agent and model not contacted with the agent is less than the difference (including, for example, absolute and/or percentage difference) in the levels of the particular Aβ peptide in test and control models. In particular embodiments, the extent to which the agent alters the levels of one or more other Aβ peptides (i.e., the peptides that are not targeted for modulation) is less than about 40%, 35%, 30%, 25%, or 20%. In one embodiment, the extent to which the agent alters the levels of one or more other Aβ peptides (i.e., the peptides that are not targeted for modulation) is less than 20%. Any modulation of the

level of the one or more other Aβ peptides (i.e., the peptides that are not targeted for modulation) that is not a substantial alteration is one that is generally not associated with any significant undesired or adverse consequence in the model cell or organism.

In a particular embodiment, agents that more specifically or selectively modulate Aβ levels can be identified in a disease model using methods provided herein that involve identifying agents that modulate Aβ levels without substantially altering or affecting non-APP substrate cleaving/processing activity of presenilin. In one embodiment, the agent that reduces Aβ42 levels does not substantially alter the levels of non-APP substrate cleavage/processing activity of presenilin, such as LRP and/or other substrates provided herein. In anther embodiment the agent that reduces Aβ42 levels does not substantially alter the levels of Aβ40 or the non-APP substrate cleavage/processing activity of presenilin. The process of further identifying an agent that does not substantially alter the cleavage of a presenilin substrate (other than APP), or portion(s) thereof, can be carried out by any of the methods described herein. In general, this process can involve a comparison of the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of a presenilin substrate (or portion(s) thereof) other than APP, and/or the levels of a peptide fragment(s) of the presenilin substrate, in a model cell or cells within a model organism that has been contacted with the test agent (i.e., test model) and in a model cell or cells within a model organism that has not been contacted with the test agent (i.e., control model). If the cleavage and/or processing of the presenilin substrate that is other than APP and/or the substrate fragment(s) levels of the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of the presenilin substrate, or portion(s) thereof, that is other than APP. The control model can be the same physical model as the test model or a different model. When the control and test models are the same, the control is the model in the absence of test agent.

G. Methods for Treating or Preventing Diseases or Disorders

Methods provided herein for identifying or screening for agents that modulate Aβ levels and for candidate agents for the treatment or prophylaxis of disease are useful in

the discovery of particular agents for treating diseases and disorders involving or characterized by altered Aβ production, catabolism, processing and/or levels. Sueh diseases include, but are not limited to, diseases involving or associated with amyloidosis and neurodegenerative diseases. One example of such a disease is Alzheimer's disease.

Provided herein are methods for treating or preventing diseases and disorders involving or characterized by altered Aβ production, catabolism, processing and/or levels. The methods are particularly suitable for the treatment or prevention of disease because they are designed to selectively modulate Aβ levels, and in particular, the level of Aβ42 and/or Aβ39, in order to avoid possible side-effects that non-specific modulation of Aβ can be associated with as described herein. Such methods can include a step of administering to a subject having such a disease or disorder or predisposed to such a disease or disorder an agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides. In one embodiment of the methods, the agent being administered is one that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides such that Aβ42 levels are modulated. The level of Aβ42 can be modulated to a greater extent than the level of one or more other Aβ peptides, in particular, Aβ40, is modulated, or without substantially altering the level of one or more other Aβ peptides, in particular Aβ40. In a particular embodiment, Aβ42 levels are reduced.

In another embodiment, the agent being administered is one that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides such that Aβ39 levels are modulated. The level of Aβ39 can be modulated to a greater extent than the level of one or more other Aβ peptides, in particular, Aβ40, or without substantially altering the level of one or more other Aβ peptides, in particular Aβ40. In a particular embodiment, the agent increases the level of Aβ39. The agent can be one that modulates the levels of Aβ42 and Aβ39 to a greater extent than the level of one or more other Aβ peptides, in particular, Aβ40, or without substantially altering the levels of one or more other Aβ peptides, such as, for example, Aβ40. In one embodiment, the levels of Aβ42 are

reduced and the levels of Aβ39 are increased.

In another embodiment of the methods, the agent being administered is one that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP. In a further embodiment, the agent being administered is one that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof (*e.g.*, Notch, E-cadherin, Erb-B4, and portion(s) thereof) that is other than APP. In another embodiment, the agent being administered is one that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof. In particular of these embodiments, the levels of Aβ42 and/or Aβ39 are modulated, such as, for example, as follows: the levels of Aβ42 and/or Aβ39 are modulated to a greater extent than the levels of other Aβ peptides, such as, *e.g.*, Aβ40; the levels of Aβ42 and/or Aβ39 are modulated without substantially altering the level of one or more other Aβ peptides, such as, *e.g.*, Aβ40. In particular embodiments of these methods, the level of Aβ42 is reduced and/or the level of Aβ39 is increased.

H. Methods of Modulating Aβ

Provided herein are methods for modulating Aβ levels. In a particular embodiment, the methods are for selectively modulating Aβ levels. The methods can be practiced to modulate Aβ levels in any sample. Examples of samples in which Aβ levels may be modulated include, but are not limited to, cells, tissues, organisms, lysates, extracts and membrane preparations of cells and cell-free samples, such as, for example, samples containing APP and/or portion(s) thereof.

Modulation of Aβ can be, for example, any alteration or adjustment that results in a change in Aβ levels, including but not limited to, alteration of Aβ levels in the cell cytoplasm, intracellular organelles, cell membranes, extracellular medium, tissue, body

fluid and/or levels of secreted Aβ. Modulation of Aβ can involve an alteration in APP (and/or portion(s) thereof) cleavage or processing, Aβ cleavage or processing and/or any combination thereof. Altered APP cleavage or processing and/or altered Aβ cleavage or processing may be the result of an alteration in any cell, organelle, enzyme, protein, and/or factor that facilitates or participates in APP cleavage or processing and/or Aβ cleavage or processing. Cells, organelles, enzymes, proteins and factors that facilitate or participate in APP cleavage or processing and/or Aβ cleavage or processing may include, but are not limited to microglial cells, proteases, such as secretases, including α, β, and γ-secretases, peptidases, presenilins, degratory enzymes, including insulin-degrading enzyme (IDE), neprilysin, plasmin, uPA/tPA, endothelin converting enzyme-1, matrix metalloproteinase-9, and proteosome, cell surface receptors, including scavenger receptor A, the receptor for advanced glycation endproducts (RAGE), and the low-density lipoprotein receptor-related protein (LRP). Modulation of Aβ can also involve an alteration in receptor-mediated clearance and/or uptake into organelles capable of processing Aβ for degradation, including, for example, endosomes and lysosomes. Modulation of Aβ levels may thus involve modulating the level, functioning and/or activity of one or more cells, organelles, enzymes, proteins, and/or factors involved in modulating Aβ production, catabolism, processing and/or clearance.

Modulation of Aβ levels can be, for example, a complete or nearly complete elimination of the production of one or more forms of Aβ, a reduction in the production of one or more forms of Aβ, or an increase in the production of one or more forms of Aβ. A modulation of Aβ can also be an increase in clearance and/or degradation of one or more forms of Aβ, or a decrease in the clearance and/or degradation of one or more forms of Aβ. Modulation of Aβ can further be an alteration in the levels of different Aβ peptides relative to one another or to the total Aβ. Thus, for example, the ratio of a particular Aβ peptide to the total Aβ in a sample can be altered in modulation of Aβ. A modulation of Aβ can also be an increase in one or more forms of Aβ concurrent with a decrease in one or more other forms of Aβ.

In particular methods for modulating Aβ provided herein, the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ

and/or the levels of Aβ is/are modulated in a manner such that Aβ levels are modulated while avoiding substantial or significant alterations in other processes, activities, mechanisms and/or compositions that are not necessary to modulate in order to modulate Aβ levels. Such modulation can be a selective or specific modulation of Aβ levels.

In one embodiment, the method selectively modulates the level of particular Aβ peptides, for example one Aβ peptide or two Aβ peptides. In a particular embodiment, the method includes a step of modulating the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the levels of Aβ such that the level of Aβ42 is modulated to a greater extent than the level of one or more other Aβ peptides, such as, *e.g.*, Aβ40 (or an Aβ peptide having a C-terminal end that terminates before amino acid 40, or an Aβ with an N-terminus cleaved after amino acid 49 (close to the cytoplasmic side of the transmembrane domain)) is modulated. The level of Aβ42 can be modulated without substantially altering the level of one or more other Aβ peptides, such as, *e.g.*, Aβ40. In a particular embodiment of these methods, the level of Aβ42 is reduced; in other embodiments, level of Aβ42 is increased. In another particular embodiment, the level of Aβ39 (or the level of one or more Aβ peptides having a C-terminal end that terminates before amino acid 40) is to a greater extent than the level of one or more other Aβ peptides, such as, *e.g.*, Aβ40, is modulated. The level of Aβ39 can be modulated without substantially altering the level of one or more other Aβ peptides, such as, *e.g.*, Aβ40. In a particular embodiment of these methods, the level of Aβ39 is increased; in other embodiments, level of Aβ39 is reduced. In particular embodiments of any of these methods, the level of the particular Aβ peptide, such as Aβ42 or Aβ39, can be changed by greater than or equal to about 50%. In one embodiment, Aβ42 levels of the sample are reduced by greater than or equal to about 50%.

In another embodiment of the methods for modulating Aβ levels, the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the levels of Aβ such that the level of Aβ42 and the level of Aβ39 are modulated to a greater extent than the level of one or more other Aβ peptides, such as, for example, Aβ40. In a further embodiment, the level of Aβ42 and the level of Aβ39 are modulated

without substantially altering the level of one or more other Aβ peptides, such as, for example, Aβ40. In a particular embodiment of these methods, the level of Aβ42 is reduced and the level of Aβ39 is increased.

In particular embodiments of these methods, the sample contains APP and/or portion(s) thereof. Samples that can be used include, but are not limited to, a cell, tissue, organism, cell or tissue lysate, cell or tissue extract, body fluid, cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample. In a particular embodiment, the sample contains a cell, including, for example, a eukaryotic cell such as a mammalian cell. Particular examples of mammalian cells include rodent or human cells. In particular embodiments, the Aβ is cellular and/or extracellular Aβ.

In other embodiments of the methods for modulating Aβ levels, the method includes a step of modulating the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering (a) one or more presenilin-dependent activities other than the presenilin-dependent processing of APP, (b) the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP and/or (c) the cleavage and/or processing of LRP and/or portion(s) thereof. In particular embodiments of these methods, the levels of Aβ42 are modulated. For example, the levels of Aβ42 may be modulated to a greater extent than the levels of other Aβ peptides, such as, for example, Aβ40. The levels of Aβ42 may be modulated without substantially altering the level of one or more other Aβ peptides, such as, *e.g.*, Aβ40. In any of these embodiments, the level of Aβ42 can be reduced or increased. In particular embodiments of these methods, the levels of Aβ39 are modulated. For example, the levels of Aβ39 may be modulated to a greater extent than the levels of other Aβ peptides, such as, for example, Aβ40. The levels of Aβ39 may be modulated without substantially altering the level of one or more other Aβ peptides, such as, *e.g.*, Aβ40. In any of these embodiments, the level of Aβ39 can be reduced or increased. In further embodiments, the levels of Aβ42 and Aβ39 are modulated. For example, the levels of Aβ42 and Aβ39 can be modulated to a greater extent than the levels of other Aβ peptides, such as, *e.g.*, Aβ40.

The levels of Aβ42 and Aβ39 levels can be modulated without substantially altering the level of one or more other Aβ peptides, such as, *e.g.*, Aβ40. In particular embodiments, the level of Aβ42 is reduced and/or the level of Aβ39 is increased. In other embodiments, the level of Aβ42 is increased. In other embodiments, the level of Aβ39 is decreased. In particular embodiments of any of these methods, the level of the particular Aβ peptide, such as Aβ42 or Aβ39, can be changed by greater than or equal to about 50%. In one embodiment, Aβ42 levels of the sample are reduced by greater than or equal to about 50%.

The sample used in these methods can be any sample, such as those described herein. For example, the sample can contain a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and/or a cell-free extract or other cell-free sample. The sample can contain presenilin (and/or portion(s) thereof), APP (and/or portion(s) thereof), and/or one or more presenilin substrates (and/or portion(s) thereof). In particular embodiments, the sample contains one or more of: LRP, Notch, E-cadherin, TrkB, APLP2, hIre1α, Erb-B4, portion(s) of LRP, portion(s) of Notch, portion(s) of E-cadherin, portion(s) of TrkB, portion(s) of APLP2, portion(s) of hIre1α, and portion(s) of Erb-B4. In particular embodiments, the sample contains a cell, such as, for example, a eukaryotic cell, including, for example, a mammalian cell. Particular examples of mammalian cells include rodent and human cells. In any of the methods, the Aβ can be cellular and/or extracellular Aβ.

In particular embodiments of the methods for modulating Aβ levels that include a step of modulating the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP, the presenilin substrate and/or portion(s) thereof, can be one or more of the following: Notch, E-cadherin, Erb-B4, and portions of Notch, E-cadherin and Erb-B4. In such embodiments, the modulation can be such that the levels of an intracellular carboxyl domain fragment of Notch, E-cadherin and/or Erb-B4 are substantially unchanged.

In particular embodiments of the methods for modulating Aβ levels that include a step of modulating the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof, the modulation can be such that the level and/or presence or absence of one or more fragments of LRP (and/or a portion(s) thereof) is substantially unchanged. In one embodiment the presence, absence and/or level of an ~20 kD fragment of LRP is substantially unchanged. The fragment can be one that (a) contains an amino acid sequence that is contained within a transmembrane region of LRP, (b) binds with an antibody generated against a C-terminal amino acid sequence of an LRP (*e.g.*, the C-terminal 13 amino acids of an LRP), (c) contains an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10, (d) is present when an LRP is not cleaved by a presenilin-dependent activity, and/or (e) occurs in the presence of an inhibitor (*e.g.*, DAPT) of a presenilin-dependent activity. In one embodiment, the modulation can be such that the level and/or presence or absence of one or more C-terminal fragments (CTF) of LRP (and/or a portion(s) thereof) is substantially unchanged.

In any of the methods for modulating Aβ levels provided herein, the modulating can be effected by any method, including, but not limited to, contacting a sample with an agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the levels of Aβ such that the level of one or more Aβ peptides, such as, for example, Aβ42, is modulated as described herein. An agent may be, for example, any agent identified using the methods provided herein for identifying agents that modulate Aβ. Agents include those that modulate the level, functioning and/or activity of one or more proteins involved in modulating Aβ. Proteins involved in modulating Aβ can be, for example, APP processing enzymes, Aβ processing enzyme, receptors or modulatory proteins thereof. In particular examples, the concentration of the agent is less than or equal to about 35 μM, 30 μM, 25 μM, 20 μM, 15 μM or 10 μM. For example, the concentration of agent is less than or equal to about 30 μM. In one embodiment, the agent reduces Aβ42 levels with an IC_{50} of about 25 μM

or less or about 20 μM or less.

I. Antibodies and Proteins that bind Aβ

Provided herein are antibodies and methods of preparing antibodies which are specifically reactive with Aβ. Also provided are proteins engineered to bind Aβ. Such antibodies and Aβ binding proteins can be used in applications such as, but not limited to, diagnostic purposes, research purposes, and in treatment of Aβ-related diseases and conditions. For example, Aβ binding proteins can be used as reagents for the assays and kits described herein for the detection of the modulation or processing of APP. Antibodies and antibody fragments described herein for use in immunological detection of Aβ, such as those used in assays to monitor APP processing and modulation, can also be used in other applications such as diagnostic purposes, research purposes, and in treatment of Aβ-related diseases and conditions. Aβ binding proteins including Aβ antibodies can also be used as candidate agents as described herein for modulating Aβ levels.

1. Aβ Antibodies

Aβ antibodies provided herein are specifically reactive with Aβ. In one embodiment, antibodies which are specifically reactive with Aβ recognize the N-terminal region of Aβ. Antibodies which recognize the N-terminal region of Aβ can be prepared by immunizing a host animal with a peptide containing the sequence of the N-terminal region of Aβ. For example, a peptide containing the sequence of amino acids 1-12 of SEQ ID NO: 4 or a fragment thereof is used to immunize mice and generate monoclonal antibodies as described herein or by method known in the art. An exemplary antibody is the Aβ antibody Aβ1-12, referred to herein as B436.

In another embodiment, antibodies are prepared which recognize only a particular Aβ or a selective number of Aβ peptides. Antibodies can be prepared by immunizing a host animal such as a mouse with portions of Aβ specific for the species of interest. For example, as described herein, antibodies can be generated which recognize only Aβ42 with minimal or no binding to other Aβ peptides, such as Aβ40. An Aβ antibody selective for Aβ42 can have at least about 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity

for Aβ42 relative to other forms of Aβ, such as Aβ40. In addition, the antibody can have an affinity constant for binding to Aβ42 of at least about 10^5 l/mol, 2 x 10^5 l/mol, 3 x 10^5 l/mol, 4 x 10^5 l/mol, 5 x 10^5 l/mol, 6 x 10^5 l/mol, 7 x 10^5 l/mol, 8 x 10^5 l/mol, 9 x 10^5 l/mol, 10^6 l/mol, 2 x 10^6 l/mol, 3 x 10^6 l/mol or 4 x 10^6 l/mol or more. An exemplary antibody is the Aβ antibody selective for Aβ42, referred to herein as A387.

Aβ antibodies can be produced which recognize some or all forms of Aβ, for example Aβ in soluble form, such as in low molecular weight forms, in plaques and in neurofibrillary tangles. Aβ antibodies can also be produced which recognize only a specific Aβ, for example Aβ42, and in some cases are also specifically reactive with specific forms of Aβ, for example Aβ42 in soluble form such as Aβ42 in plasma and Aβ42 in low molecular weight forms. Aβ antibodies which recognize only specific Aβ peptides, and/or are specifically reactive with specific forms of Aβ can be used to ascertain the form(s) and types of Aβ peptides in a sample, for purposes of diagnosis, such as in methods described herein or known in the art. Such Aβ antibodies can be used for treatment for example, where a predominant form and/or Aβ peptide is associated with an Aβ-related condition or the modulation of a form and/or a particular Aβ is effective for treatment.

Antibodies can be prepared using a variety of methods well-known in the art. For example, as described herein, a target epitope such a peptide, peptide fragment or synthetic peptide may be prepared and used to immunize a host animal. As further described herein, monoclonal antibodies can be prepared, cell lines producing monoclonal antibodies can be isolated and the nucleic acid sequence encoding the monoclonal antibodies as well as the amino acid sequence of the antibodies can be obtained.

An antibody can be any derivative of an immunoglobulin. Aβ antibodies include antibodies that are less than full-length, *e.g.* antibody fragments, retaining at least a portion of the full-length antibody's specific binding ability. Examples of such antibodies include, but are not limited to, Fab, Fab', $F(ab)_2$, single-chain Fvs (scFv), Fv, dsFv and diabody fragments. Antibodies can include multiple chains linked together, such as by disulfide bridges. Antibodies can be prepared enzymatically and by

recombinant DNA technology.

(a) **Fab and $F(ab)_2$ fragments**

Fab fragments are antibody fragments that can be produced from digestion of an immunoglobulin with papain. A Fab fragment contains a complete light chain paired with the variable region and the C_H1 region of the heavy chain. Recombinant means such as expression in a host cell, synthetic production or *in vitro* expression systems can also be used to produce Fab fragments of similar or equivalent structure to Fab fragments produced by enzymatic digestion .

Fab fragments can be generated which are specifically reactive with Aβ or with particular Aβ peptides. In one embodiment, an Fab recognizes all or most Aβ peptides. For example, an Fab is produced which recognizes the N-terminal amino acids of Aβ such as an Fab generated from the antibody B436 or an Fab produced using the sequence or a portion of the sequence of the B436 antibody.. In another embodiment, an Fab is specifically reactive with a specific Aβ, for example, Aβ42.

Fab fragments can be produced by enzymatic means. For example, an Fab can be generated from Aβ antibodies such as A387 and/or B436 by isolating immunoglobulin from antibody producing cells, such as described in the examples herein or by methods known in the art. Fab antibodies are generated by cleaving the A387 and/or B436 immunoglobulin molecules with papain.

In another embodiment, Fab molecules are generated from Aβ antibodies such as A387 and/or B436 by recombinant means using the sequences of the light and heavy chain variable regions and mimicking the papain cleavage by constructing the polypeptides of the heavy and light chain variable domains to have the same or similar (within 1 or more amino acids in length difference) amino acid sequences. For example, A387 Fab molecules can be constructed containing the amino acid sequences or a portion thereof, of SEQ ID NOs: 12 and 14. In one aspect of the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:11 and/or 13 are used to construct an Fab antibody. B436 Fab molecules can be constructed containing the amino acid sequences or a portion thereof, of SEQ ID NOs: 16 and 18. In one aspect of the embodiment, nucleic acid molecules containing the sequence or a portion thereof

of SEQ ID NO:15 and/or 17 are used to construct an Fab antibody.

An $F(ab)_2$ fragment is an antibody fragment that can be produced from digestion of an immunoglobulin with pepsin at pH 4.0-4.5. An $F(ab)_2$ fragment contains both light chains associated with the variable regions and the C_H1 regions of the two heavy chains. Disulfide bridges link the two antigen binding arms of the $F(ab)_2$ fragment. Recombinant means such as expression in a host cell, synthetic production or *in vitro* expression systems can also be used to produce $F(ab)_2$ fragments of similar or equivalent structure to $F(ab)_2$ fragments produced by enzymatic digestion.

$F(ab)_2$ fragments can be produced which are specifically reactive with $A\beta$ and/or specific $A\beta$ peptides. In one embodiment, an $F(ab)_2$ fragment recognizes the N-terminal amino acids of $A\beta$ such as an $F(ab)_2$ from the antibody B436 or an $F(ab)_2$ produced using the sequence or a portion of the sequence of the B436 antibody. In another embodiment, an $F(ab)_2$ is specifically reactive with a specific $A\beta$, for example, $A\beta42$. For example, an $F(ab)_2$ is generated from the antibody A387 or an $F(ab)_2$ is produced using the sequence or a portion of the sequence of the A387 antibody.

$F(ab)_2$ fragments can be produced by enzymatic means. For example, an $F(ab)_2$ can be generated from $A\beta$ antibodies such as A387 and/or B436 by isolating immunoglobulin from antibody producing cells, such as described in the examples herein or by methods known in the art. $F(ab)_2$ antibodies are generated by cleaving the A387 and/or B436 immunoglobulin molecules with pepsin.

In another embodiment, $F(ab)_2$ molecules are generated from $A\beta$ antibodies such as A387 and/or B436 by recombinant means using the sequences of the light and heavy chain variable regions and mimicking the pepsin cleavage by constructing the polypeptides of the heavy and light chains to have the same or similar (within 1 or more amino acids in length difference) amino acid sequences. For example, A387 $F(ab)_2$ molecules can be constructed containing the amino acid sequences or a portion thereof, of SEQ ID NOs: 12 and 14. In one aspect of the embodiment, nucleic acid moleeules containing the sequence or a portion thereof of SEQ ID NO:11 and/or 13 are used to construct an $F(ab)_2$ antibody. B436 $F(ab)_2$ molecules can be constructed containing the amino acid sequences or a portion thereof, of SEQ ID NOs: 16 and 18. In one aspect of

the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:15 and/or 17 are used to construct an $F(ab)_2$ antibody.

(b) Fv and dsFv fragments

An Fv antibody fragment is composed of one variable heavy domain (V_H) and one variable light domain linked by noncovalent interactions. Fv fragments can be generated by recombinant DNA technology produce the variable domains of the heavy and light chains, for example in a host cell, or by synthetic means. In one embodiment, an Fv fragment is generated from the A387 by recombinant means using nucleotide sequences encoding the heavy chain and light chain variable domains set forth in SEQ ID NO:12 and 14. In one aspect of the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:11 and/or 13 are used to construct an Fv fragment.

In another embodiment, Fv fragments are generated by recombinant means using nucleotide sequences encoding the heavy chain and light chain variable domains set forth in SEQ ID NO:16 and 18. In one aspect of the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:15 and/or 17 are used to construct an Fv fragment.

A dsFV refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the V_H-V_L pair. Chain dissociation may be prevented by introducing Cys residues at appropriate locations into the framework of V_H and V_L in order to form a disulphide crosslink (Glockshuber *et al.*, 1990; Reiter *et al.*, 1996). dsFv molecules can be generated by recombinant means to produce dsFv antibodies from A387 and/or B436. For example, cysteines can be engineered into the sequence of the heavy and light chains to provide a disulfide bond between them. dsFvs can then be generated by enzymatic or by recombinant means.

(c) ScFvs and diabodies

scFvs refer to antibody fragments that contain a variable light chain (V_L) and variable heavy chain (V_H) covalently connected by a polypeptide linker in any order. The linker is of a length such that the two variable domains are bridged without substantial interference. Included linkers are $(Gly\text{-}Ser)_n$ residues with some Glu or Lys residues

dispersed throughout to increase solubility. scFvs are generated by recombinant means and may be produced synthetically, *in vivo*, such as by expression in a host cell or transgenic organism, or using *in vitro* systems known in the art. scFvs can be advantageous because of the smaller size.

scFvs can be generated which are specifically reactive with to Aβ or to specific Aβ peptides. In one embodiment, an scFv is produced which recognizes the N-terminal region of Aβ. For example, an scFv is generated using the sequence of the antibody B436 or a portion thereof, such as the sequence comprising the variable regions of the heavy and light chain of B436. A linker region is used such as those described herein or known in the art to join the variable regions. In another embodiment, an scFvs is generated which recognizes specific Aβ peptide, for example, an scFV which are specifically reactive with Aβ42. In one embodiment, an scFv is generated using the sequence of the antibody A387 or a portion thereof, such as the sequence comprising the variable regions of the heavy and light chain of A387. A linker region is used such as those described herein or known in the art to join the variable regions. For example, an scFv is generated containing the sequence of amino acids or a portion thereof of SEQ ID NO:12 and/or 14. In another embodiment, an scFv is generated using the sequence of the antibody B436 or a portion thereof, such as the sequence comprising the variable regions of the heavy and light chain of B436. A linker region is used such as those described herein or known in the art to join the variable regions. For example, an scFv is generated containing the sequence of amino acids or a portion thereof of SEQ ID NO:16 and/or 18.

(d) Complementarity-determining Regions (CDRs)

Complementarity-determining regions (CDRs) (also referred to as hypervariable regions) refer to regions of an immunoglobulin molecule that vary greatly in amino acid sequence relative to flanking Ig sequences. The length and conformation of CDRs vary among Igs, but generally CDRs form short loops supported by a sandwich of two anti-parallel beta-sheets within the variable regions of the antibody. Three CDRs, designated CDR-L1, CDR-L2 and CDR-L3, are present in the variable region of an immunoglobulin light chain, and three CDRs, designated CDR-H1, CDR-H2 and CDR-H3, are present in the variable region of an immunoglobulin heavy chain. Each CDR generally contains at

least one, and often several, amino acids residues that make contact with antigen, but all six CDRs are not necessarily required to maintain the binding specificity of an antibody.

Several definitions of CDRs are commonly in use, and CDRs identified according to the different definitions generally overlap, but may differ slightly in their boundaries. The Kabat CDR definition is based on sequence variability among immunoglobulins. The Chothia CDR definition is based on the location of structural loop regions. The AbM CDR definition is a compromise between the Kabat and Chothia definitions used by Oxford Molecular's AbM antibody modeling software. The contact CDR definition is based on a comparison of the available complex crystal structures.

Taking into account these alternative CDR definitions, some general principles have been devised to identify CDRs based on a given amino acid sequence are shown in Table 3 (see, for example, www.bioinf.org.uk/abs/).

Table 3

	Start	**Residue(s) before**	**Residue(s) after**	**Length**	**Alternatives**
CDR-L1	~ residue 24	Usually C	Usually W	~10-17 residues	
CDR-L2	Usually 16 residues after end of L1	Usually I-Y, V-Y, I-K or I-F		Usually 7 residues	
CDR-L3	Usually 33 residues after end of L2	Usually C	Usually F-G-X-G	~7-11 residues	
CDR-H1	~ residue 26	Usually C-X-X-X	Usually W	~10-12 residues	Kabat definition starts 5 residues later (length is ~5-7 residues)

					Chothia definition ends 4 residues earlier (length is ~6-8 residues)
CDR-H2	Usually 15 residues after end of H1	Often similar to L-E-W-I-G	Often K/R/L-L/I/V/F/A-T/S/I/A	~16-19 residues	AbM and Chothia definitions end 7 residues earlier (length is ~9-12 residues)
CDR-H3	Usually 33 residues after end of H2	Usually C-X-X	Usually W-G-X-G	~3-25 residues	

Applying these principles to the antibody sequences disclosed herein, exemplary CDR sequences of the A387 and B436 antibodies can be defined as shown in Table 4.

Table 4

	Exemplary CDR sequence	**Exemplary CDR sequences according to alternative CDR definition(s)**
A387 CDR-L1	RASQSISNNLH (aa 24-34 of SEQ ID NO:12)	
A387 CDR-L2	YASQSIY (aa 50-56 of SEQ ID NO:12)	
A387 CDR-L3	QQSHSWPLT (aa 89-97 of SEQ ID NO:12)	
A387 CDR-H1	GFTFSNDAMS (aa 26-35 of SEQ ID NO:14)	NDAMS (Kabat) (aa 31-35 of SEQ ID NO:14) GFTFSN (Chothia)

		(aa 26-31 of SEQ ID NO:14)
A387 CDR-H2	SISSVGNTYYPDSVKG (aa 50-65 of SEQ ID NO:14)	SISSVGNTY (Chothia and AbM) (aa 50-58 of SEQ ID NO:14)
A387 CDR-H3	GYGVSPWFSY (aa 98-107 of SEQ ID NO:14)	
B436 CDR-L1	RSSQNIVHSSGNTYLE (aa 24-39 of SEQ ID NO:16)	
B436 CDR-L2	KVSNRFS (aa 55-61 of SEQ ID NO:16)	
B436 CDR-L3	FQGSHVPYT (aa 94-102 of SEQ ID NO:16)	
B436 CDR-H1	GFTFSRYTMS (aa 26-35 of SEQ ID NO:18)	RYTMS (Kabat) (aa 31-35 of SEQ ID NO:18) GFTFSR (Chothia) (aa 26-31 of SEQ ID NO:18)
B436 CDR-H2	TINFGNGNTYYPDSVKG (aa 50-66 of SEQ ID NO:18)	TINFGNGNTY(Chothia and AbM) (aa 50-59 of SEQ ID NO:18)
B436 CDR-H3	LNWAY (aa 99-103 of SEQ ID NO:18)	

Thus, as used herein, a "CDR of antibody A387" refers to a sequence of amino acids that is a) the same as one of the amino acid sequences set forth in rows 2-7 of Table 4; b) a fragment of SEQ ID NO:12 or 14 with N- and/or C-terminal boundaries that differ by no more than about 4, 3, 2 or 1 amino acids relative thereto; or c) is at least 60%, 65%, 70%, 80%, 85%, 90%, 95% or more identical to a) or b). A CDR of antibody A387 also includes substitutions within the amino acid sequences of the CDRs set forth in rows 2-7 of Table 4 that when substituted into an A387 antibody do not substantially alter the binding affinity or selectivity of the antibody as compared with the unmodified A387

antibody. Such substitutions can be conservative amino acid substitutions (for example, conservative amino acid changes set forth in Table 2). Generally such substitutions can be for example, 1 amino acid change or 2 amino acid changes within a CDR sequence set forth in rows 2-7 of Table 4.

As used herein, a "CDR of antibody B436" refers to a sequence of amino acids that is a) the same as one of the amino acid sequences set forth in rows 8-13 of Table 4 b) a fragment of SEQ ID NO:16 or 18 with N- and/or C-terminal boundaries that differ by no more than about 4, 3, 2 or 1 amino acids relative thereto; or c) is at least 60%, 65%, 70%, 80%, 85%, 90%, 95% or more identical to a) or b). A CDR of antibody B436 also includes substitutions within the amino acid sequences of the CDRs set forth in rows 8-13 of Table 4 that when substituted into a B436 antibody do not substantially alter the binding affinity or selectivity of the antibody as compared with the unmodified B436 antibody. Such substitutions can be conservative amino acid substitutions (for example, conservative amino acid changes set forth in Table 2). Generally such substitutions can be for example, 1 amino acid change or2 amino acid changes within a CDR sequence set forth in rows 8-13 of Table 4.

One or more, up to all of the CDRs of an $A\beta$ antibody can be used to bind $A\beta$ or a specific form of $A\beta$. The CDRs may be produced by recombinant means such as produced synthetically, *in vivo*, such as by expression in a host cell or transgenic organism, or using *in vitro* systems known in the art. CDRs may be produced as isolated sequences or may comprise a portion of a larger molecule such as an immunoglobulin, an Fab, $F(ab)_2$, an scFv , diabody or a chimeric polypeptide. Multimerization of antibody fragments or antibody domains can be used increase the avidity of such molecules for $A\beta$ and/or specific $A\beta$ peptides and/or forms of $A\beta$. Chemical means, such as by crosslinking or disulfide bond formation can be used to generate multimeric forms of antibodies. Recombinant means can also be used, for example by constructing repetitive domains or by introducing functionalities which can then be used for cross-linking or association by other means.

2. Engineering $A\beta$ binding proteins

Antibodies or regions thereof, such as CDRs, can be engineered to generate $A\beta$

binding proteins which bind Aβ or particular peptides or forms of Aβ. For example Aβ binding proteins can be engineered to optimize the binding to Aβ and/or a particular Aβ and/or specific forms of Aβ, to optimize attributes for specific uses such as treatment or diagnostic methods, optimize attributes for production or other desirable characteristics.

In one embodiment, an Aβ binding protein is generated which binds to a particular Aβ and/or binds selectively to one or more Aβ peptides. For example, an Aβ binding protein is engineered to retain substantially the same binding properties as an Aβ antibody. In one embodiment, an Aβ binding protein is engineered to retain substantially the same binding properties as the A387 antibody. In another embodiment, an Aβ binding protein is engineered to retain substantially the same binding properties as the B436 antibody.

As described herein, Aβ binding proteins can be generated which recognize only Aβ42 with minimal or no binding to other Aβ peptides, such as Aβ40. An Aβ binding protein selective for Aβ42 can have at least about 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity for Aβ42 relative to other forms of Aβ, such as Aβ40. In addition, the Aβ binding protein can have an affinity constant for binding to Aβ42 of at least about 10^5 l/mol, 2 x 10^5 l/mol, 3 x 10^5 l/mol, 4 x 10^5 l/mol, 5 x 10^5 l/mol, 6 x 10^5 l/mol, 7 x 10^5 l/mol, 8 x 10^5 l/mol, 9 x 10^5 l/mol, 10^6 l/mol, 2 x 10^6 l/mol, 3 x 10^6 l/mol or 4 x 10^6 l/mol or more.

Aβ binding proteins can be generated for example, from portions of antibodies that recognize Aβ can be engineered into other protein scaffolds. Nucleic acid molecules encoding such portions along with nucleic acid molecules encoding scaffolds can be used to construct Aβ binding proteins including Aβ antibodies using standard molecular biology techniques known to one skilled in the art. Exemplary nucleic acid molecules include but are not limited to SEQ ID NOs. 11, 13, 15, 17, 97, 98, 99 and 100. Additionally, nucleic acid molecules can be generated by reverse translating Aβ binding protein amino acid sequences. For example, a nucleic acid sequence is derived from a portion of an Aβ antibody, such as a CDR amino acid sequence. There are a number of possible nucleic acid sequences based on the degeneracy of codons which can be used for

each amino acid. However, for the purposes of constructing Aβ binding proteins, any nucleic acid sequence which encodes the amino acid sequence can be used for constructing an Aβ binding protein. Nucleic acid molecules encoding Aβ binding proteins, antibodies or portions thereof can be mutagenized to alter binding characteristics. Additional functionalities such as detectable moiety or a therapeutic moiety can be added to Aβ binding proteins and antibodies. Protein and peptide chemistry can also be used to construct Aβ binding proteins.

(a) Scaffolds

A scaffold refers to a structure that forms a conformationally stable structural support, or framework, which is able to display one or more sequences of amino acids, such as a CDR, a variable region or a binding domain, in a localized surface region. A scaffold may be a naturally occurring polypeptide or polypeptide "fold" (a structural motif), or may have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a naturally-occurring polypeptide or fold. A review of protein scaffolds and their uses can be found in Skerra (2000) *J. Mol. Recognition* 13:167-187.

i. Antibody Scaffolds

Immunoglobulins comprise a natural type of biomolecular scaffold. Aβ binding proteins can be engineered based on immunoglobulin molecules or portions thereof including, CDR grafting, humanized antibodies, single Ig and Ig-like scaffolds and antibody fragments such as Fvs, scFvs, Fabs, and $F(ab)_2$s.

Accordingly, provided herein are antibodies and antibody fragments for use as antibody scaffolds. Such scaffolds can contain the heavy and/or light chains of an immunoglobulin or portions thereof. In one embodiment, an antibody scaffold is constructed from a heavy chain. The heavy chain can be from an Aβ antibody such as from A387 or B436 or from any heavy chain known in the art. In another embodiment, an antibody scaffold is constructed from the constant region of one antibody and the variable region from an Aβ antibody. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NOs: 69, 71, 83, 85 or 87 and the variable

region can contain the amino acids of SEQ ID NO 14 or 18 or a portion thereof. A joining region can be used from either an Aβ antibody or from an antibody known in the art. Exemplary joining regions are described herein. In a particular embodiment, an antibody scaffold contains a variable region containing the sequence of amino acids 1-97 of SEQ ID NO:14 or 1-98 of SEQ ID NO: 18. In another embodiment, an antibody scaffold is constructed from a light chain. The light chain may be from an Aβ antibody or from any light chain known in the art. In another embodiment, an antibody scaffold is constructed from the constant region of one light chain and the variable region from an Aβ antibody. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NOs: 63, 65 or 81 and the variable region can contain the amino acids of SEQ ID NO 12 or 16, or a portion thereof. A joining region can be used from either an Aβ antibody or from an antibody known in the art. Exemplary joining regions are described herein. In a particular embodiment, an antibody scaffold contains a variable region containing the sequence of amino acids 1-95 of SEQ ID NO:14 or 1-100 of SEQ ID NO:16. Heavy and light chains can also be constructed containing a portion of an antibody known in the art and a portion of an Aβ antibody, for example by grafting the variable domain of an Aβ heavy chain, the DJ region and a portion of the C domain to another heavy chain containing the remainder of the C domain, thereby reconstructing a heavy chain. In another example, a light chain can be constructed by the variable domain of an Aβ light chain, the J region and a portion of the C domain to another light chain containing the remainder of the C domain, thereby reconstructing a light chain

Antibody scaffolds can be constructed for Fab, F(ab)2, Fvs, dsFvs, diabodies and other antibodies by methods as described herein or known in the art. Scaffolds for antibodies can also be constructed by utilizing other antibodies known in the art and altering the binding specificity such that antibodyrecognizes Aβ. For example, the variable region or a portion thereof can be grafted onto the antibody or used to replace the equivalent region within the scaffold. Single CDR regions can be grafted and/or used for replacement as well as all of the CDR regions of the light chain and/or heavy chain or any combination thereof. Mutagenesis can also be used to alter the binding specificity of an existing antibody such that it binds Aβ.

Antibody scaffolds can also be used to generate antibodies with the specificity from one antibody and the properties of another, such as reduced immunogenicity when administered in a particular animal species. Monoclonal antibodies are most often generated in non-human species, such as mice. Humanized antibodies can be generated where at least one portion of the antibody structure is of human origin. For example, a humanized antibody can be comprised of the antigen binding regions from an antibody generated in a mouse with the remainder of the antibody framework derived from a human antibody (see, for example, Hurle and Gross, Curr Opin Biotechnol. 1994 Aug;5(4):428-33). The generation of humanized antibodies includes the methods referred to in the art as CDR-grafting. Humanized antibodies can be prepared by synthetic methods or through recombinant DNA methods well known in the art.

Accordingly, provided herein are humanized antibodies which bind to Aβ. In one embodiment, one or more CDRs of an Aβ antibody is grafted onto a human antibody framework such as an Fab and scFv framework. For example, one or more of the CDRs of the Aβ antibody A387 is grafted onto a human antibody framework to create a humanized Aβ antibody. A387 CDRs can be any one or more than one of the CDRs listed in Table 4 for A387 including A387 CDR L1, L2, L3, H1, H2 and H3 in any combination. A387 CDRs also include fragments of the amino acid sequences set forth in SEQ ID NO:12 and SEQ ID NO:14. In another embodiment, one or more of the CDRs of the Aβ antibody B436 is grafted onto a human antibody framework. B436 CDRs can be any one or more than one of the CDRs listed in Table 4 for B436 including B436 CDR L1, L2, L3, H1, H2 and H3 in any combination. B436 CDRs also include fragments of the amino acid sequences set forth in SEQ ID NO:16 and SEQ ID NO:18. In one embodiment, the humanized antibodies contain the 6 CDRs of an Aβ antibody, for example, a humanized antibody with the 6 CDRs of A387. In another example, a humanized antibody contains the 6 CDRs of antibody B436.

Any human antibody framework known in the art can be used to prepare humanized antibodies. For example, a human framework can be a human scFv antibody, a human Fab fragment, a human light chain, a human heavy chain or a full immunoglobulin structure comprised of both a heavy and a light chain. Exemplary

human immunoglobulin regions useful in constructing scaffolds are those such as, but not limited to, polypeptides set for the in SEQ ID NOs: 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, and 91.

Additionally, a human antibody framework may be optimized for example to improve solubility properties or increase production in a host. For example, a camelized version of a human V_H domain can be constructed as a human antibody fragment or as a portion of a larger human antibody framework (see for example, Davies and Riechmann (1995) Bio/technology 13:475-479 and Davies and Riechmann (1996) Prot. Eng 9:531-537). CDR grafting can be used to engineer Aβ binding proteins in Ig chain scaffolds such as single Ig and Ig-like scaffolds. For example, camelid antibodies are heavy chain antibodies which are devoid of light chains so that their V_H domains remain soluble without dimerization. An Aβ binding protein can be constructed, for example, by grafting one or more of the CDRs of an Aβ antibody into the camelid antibody structure. Human and murine variable domains have been described, which do not depend on the association with another domain and can be used to create a single Ig-like scaffold for an Aβ binding protein. An additional small Ig-like framework is the minibody, for example, based on the heavy chain variable domain of an antibody comprising three strands from each β-sheet and having regions that structurally correspond to CDR-H1 and CDR-H2. Minibodies also generally contain a metal-binding site and solubilizing tri-lysine motifs at the N- or C-termini (Bianchi *et al.* (1994) *J. Mol. Biol.* 236:649-659). Isolated VH domains containing CDR1 and CDR2 and associated framework can also be used (Davies *et al.*,(1995) *Biotechnology* 13:475-479) CDR regions of an Aβ antibody such as the CDR-H1 and CDR-H2 regions from the A387 or B436 antibodies can be used to construct Aβ minibodies.

An example of a single Ig-like scaffold is the fibronectin type III domain (FN3) which constitutes a small, monomeric natural β-sandwich protein with resemblance to a trimmed Ig V_H domain. It possesses seven β-strands with three loops connecting the strands in a pairwise fashion at one end of the β-sheet. The loops can be replaced with one or more CDRs from an Aβ antibody to create an Aβ binding protein with a fibronectin scaffold. FN3 domains are found in numerous binding proteins, such as cell

adhesion molecules, cell surface hormone and cytokine receptors, chaperonins and carbohydrate-binding proteins, and generally contain seven β-strands with three loops connecting the strands in a pairwise fashion at one end of the β-sheet. An exemplary FN3 domain scaffold is derived from the tenth FN3 repeat in human fibronectin (Koide *et al.* (1998) *J. Mol. Biol.* 284:1141-1151; WO 98/56915; WO 02/04523). Another example of a single Ig-like domain scaffold is the V-like domain of the human cytotoxic T-lymphocyte associated protein-4 (CTLA-4) (Nutall et al. (1999) *Proteins Struct. Funct. Genet.* 36:217-227).

ii. Other Polypeptide Scaffolds

Beyond antibody scaffolds, other proteins with suitable architecture can be used as scaffolds to create Aβ binding proteins. Many of these proteins have defined folds and loops that are appropriate for insertion or replacement with Aβ binding regions such as one or more CDRs of an Aβ antibody. A scaffold may be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus or may be generated by rational design (*e.g.* an artificial scaffold).

Protease inhibitors generally have a binding site that comprises an exposed loop in a context of a structural framework that is specific for the inhibitor family and thus can be employed as a scaffold for a structurally constrained peptide loop Roberts et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:2429-2433; Markland *et al.* (1996) *Biochemistry* 35:8045-8057; McConnell and Hoess (1995) *J. Mol. Biol.* 250:460-470). Protease inhibitor scaffold include but are not limited to scaffolds from Bovine (or basic) pancreatic trypsin inhibitor, BPTI, the Kunitz domain of human lipoprotein-associated coagulation inhibitor (LACI-D1), human pancreatic secretory trypsin inhibitor (PSTI), bacterial serine protease inhibitor ecotin, and Tendamistat. The exposed loop may be replaced by one or more CDRs of an Aβ antibody to create an Aβ binding protein.

Helical bundle proteins can also be used as scaffolds (Braisted and Wells (1996) *Proc. Natl. Acad. Sci. USA* 93:5688-5692; Ku and Schultz (1995) *Proc. Natl. Acad. Sci. USA* 92:6552-6556). For example, an engineered single domain, called 'Z', of *Staphylococcal* protein A has a simple fold as a bundle of three α-helices. It is highly

soluble and stable against proteolysis and heat-induced unfolding. Another example is cytochrome b_{562}, with four-helix bundle proteins providing rigid framework and two loops, each connecting one pair of the α-helices. Artificial helical bundle scaffolds are also available. One of more CDR regions from an Aβ antibody can be grafted into the helical structure for example, into the loop regions between one or more of the helices to create an Aβ protein.

An additional scaffold is the β-barrel which is made of antiparallel β-strands winding around a central axis with loops connecting the strands at the open end of the resulting conical structure. For example, the β barrel framework of lipocalins (Muller and Skerra (1994) *Biochemistry* 33:14126-14135) may be used such as by grafting of a domain onto the solvent-exposed outer surface of the β-barrel. One or more CDRs of an Aβ antibody can be grafted onto a lipocalin scaffold. Examples of lipocalin scaffold include but are not limited to retinol-binding protein (RBP), bilin binding protein (BBP), apolipoprotein D, tear lipocalin and β-Trace, also known as prostaglandin D synthase. Many lipocalins based on their human framework and natural presence in human body fluids are suitable both for diagnostic and therapeutic purposes.

Knottins (Le Nguyen *et al.*, 1990) comprise a structural family defined by a small triple-stranded antiparallel β-sheet stabilized by an arrangement of disulphide bonds. Members of the knottin family include the trypsin inhibitor EETI-II from *Ecballium elaterium* seeds, the neuronal N-type Ca^{2+} channel blocker (ω-conotoxin from the venom of the predatory cone snail *Conus geographus*, and the C-terminal cellulose-binding domain (CBD) of cellobiohydrolase I from the fungus T. *reesei*. Loop structures within the Knottins can be used for insertion of or replaced with one or more CDR sequences to form Aβ binding proteins.

Other structural folds that may be suitable as scaffolds include TIM barrels, which are found, for example, in triose phosphate isomerase proteins (Altamirano *et al.* (2000) Nature 403:617-622); GST enzyme frameworks, pleckstrin homology domains, zinc finger domains and β-prism motifs.

Exemplary modifications to a polypeptide that may make it suitable for use as a scaffold include deletions of those regions that form binding loops in the naturally-

occurring molecule (*e.g.* deletions of the naturally-occurring binding sites); deletions of those regions that are unnecessary for structural integrity of the fold; substitutions of amino acids that flank the loop regions with residues that improve the properties of the polypeptide (such as improved affinity, specificity, or solubility; reduced immunogenicity, etc.); addition of detectable sequences, such as epitope tags; and the like.

iii. Non-polypeptide Scaffolds

Aβ antibodies and Aβ binding proteins, fragments thereof, such as a CDR, can also be displayed on a scaffold such as a solid support. Such scaffolds are useful in applications including but not limited to, diagnostic assays, screening assays, and cellular delivery of polypeptides.

Solid supports include but are not limited to membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. A solid support can be composed of any material that allows for the immobilization or attachment of molecules, such that these molecules retain their desired properties, such as binding ability. Examples of materials include silica, polymeric materials or glass. Solid supports can be used to display Aβ binding proteins, antibodies and fragments thereof, for example for screening purposes, diagnostic purposes, protein purification and binding assays. Additionally, solid supports such as beads and particles can be used to deliver Aβ binding proteins and antibodies to cells, animals and subjects. Aβ binding proteins, antibodies and fragments thereof can be associated with solid supports covalently such as by chemical linkage or by non-covalent interactions such as by charge interactions, interactions with other proteins or small molecules.

(b) Mutagenesis of Aβ binding regions

As described herein, Aβ binding proteins can be constructed from Aβ binding regions such as Aβ antibodies and antibody fragments including one or more CDRs. Properties of such Aβ binding proteins can be altered or optimized. For example properties such as binding affinity, binding specificity, solubility, aggregation and stability can be optimized for particular applications.

Mutagenesis techniques such as site-directed mutagenesis, random mutagenesis including random mutagenesis of discrete regions of Aβ binding proteins and other methods known in the art can be used to generate variations within the Aβ binding regions, or at one or more junctions between the Aβ binding regions and the scaffold. The variants can then be screened for Aβ binding by methods such as described herein or known in the art and variants with improved binding affinities or binding affinities optimized for particular applications such as diagnostics or treatment regimes can be isolated. For example, one or more CDRs of an Aβ antibody such as the CDRs of A387 and/or B436 can be mutagenized and then the variants generated are tested for Aβ binding. Random mutagenesis or directed conservative amino acid changes can be made in one or more CDRs. The variants can also be tested for selective binding to one or more specific Aβ peptides such as binding to Aβ42, or Aβ1-12. The variants can be screened to assess for their binding to specific forms of Aβ. For example, variants can be assayed for their binding to Aβ in plasma, cerebral spinal fluid (CSF), plaques, and neurofibrillary tangles as well as in low molecular weight and high molecular weight forms.

Variants can also be assessed for properties other than binding to Aβ. For example, variants can be isolated which are more soluble when produced synthetically or in a host by recombinant means. Variants can also be isolated which exhibit altered stability, for example increased stability or alternatively higher turnover. Such variants can be produced by mutagenizing regions outside the Aβ binding regions for example in the scaffold, antibody framework or other domains which are part of the Aβ binding protein. Such variants can also be produced by mutagenizing the Aβ binding regions or the entire Aβ binding protein and then screened for retention of Aβ binding as one of the criteria for selecting a variant.

(c) Clearance domains

A clearance domain directly or indirectly mediates enhanced clearance of a polypeptide from the circulation. A polypeptide containing a clearance domain will have a shorter half-life in the circulation, alone and/or when bound to Aβ, than a polypeptide without such a domain. Clearance mechanisms include receptor-mediated internalization

by specialized cells, such as macrophages or macrophage precursors, endothelial cells lining the sinusoids of the liver, spleen, and bone marrow, and reticular cells of lymphatic tissue and of bone marrow. Examples of receptors that mediate clearance of polypeptides in the circulation include Fc-γ receptor(s), which bind IgG-antigen complexes; lipoprotein receptors (e.g. LDL receptor-related protein receptor (LRP), LDL receptor and VLDL receptor); scavenger receptors (e.g. LRP, LDL-receptor, SR-A, SR-BI, CD36, etc.), which bind many different classes of serum macromolecules; hyaluronan receptors, which bind matrix proteoglycans; collagen alpha-chain receptors, which bind collagen alpha-chains; mannose receptors, which bind carboxy-terminal propeptides of type I procollagen and tissue plasminogen activator; and the like. A clearance domain can thus be a ligand for a receptor that mediates clearance, such as a polypeptide or fragment thereof that binds a receptor type mentioned above.

An example of a clearance domain is a ligand for an Fc receptor. There are several Fc receptors (FcR), including FcγRI, FcγRII, FcγRIII, and the neonatal Fc receptor (FcRn), which bind IgG antibodies. An Fc receptor ligand can be the Fc portion of an IgG (i.e. the portion containing the carboxy termini of the two heavy (H) chains, when an antibody is cleaved with papain), or a fragment thereof that retains Fc receptor binding. The antibody portions involved in Fc receptor binding are known in the art or can be determined by receptor binding assays known in the art. For example, the lower hinge and the adjacent region of the CH2 domain of IgG Fc are involved in binding to FcγRIIa, whereas the Fc CH2-CH3 interface is involved in binding to FcγRIIb and FcRn (Wines *et al.* (2000) *J. Immunol.* 164:5313-5318). Exemplary clearance domains are the Fc domain of an IgG1 human or an Fc domain of antibody IgG2a mouse antibody.

Another example of a clearance domain is a ligand for LRP. At least 30 molecules that bind LRP are known in the art, including, for example, APP, ApoE, alpha-2-macroglobulin, tPA, blood coagulation factors, lactoferrin, C1 inhibitor, pregnancy zone protein, thrombospondins, complement C3, and the like (see Herz and Strickland (2001) *J. Clin. Invest.* 108:779-784). The portions of these proteins that bind LRP are known in the art, or can be determined by LRP binding assays known in the art (see, for example, U.S. Patent No. 6,472,140, which describes LRP-binding fragments of

alpha-2-macroglobulin that comprise residues 1366-1392 of human alpha-2-macroglobulin). Any of these molecules, of portions thereof that bind LRP, can be used as clearance domains.

Provided herein are Aβ binding proteins containing a clearance domain. In one embodiment, an Aβ binding protein comprises an Aβ antibody and an Fc region. The Fc region may originate from the Aβ antibody or the Fc domain may be from another antibody or generated synthetically and joined to the Aβ antibody by recombinant or chemical means. In another embodiment, an Aβ binding protein comprises one or more CDRs from an Aβ antibody and additionally, an Fc clearance domain, for example an Aβ binding protein containing one or more CDRs of an Aβ antibody grafted into a scaffold and an Fc clearance domain. In yet another embodiment, an Aβ binding protein comprises a clearance domain from an LRP ligand.

(d) Additional functionalities

Aβ binding proteins can be constructed which comprise additional functionalities such as a moiety for detection or purification of the Aβ binding protein, a therapeutic moiety or an additional domain such as for indirect clearance.

Detectable moieties may be associated with an Aβ binding protein by chemical or recombinant means. For example, a protein domain which can be detected by visible or enzymatic assay can be coupled to an Aβ binding protein. Example of such domains include fluorescent proteins such as green, red and blue fluorescent proteins, β-galactosidase, alkaline phosphatase and others known in the art. A radiolabel may also be coupled to an Aβ binding protein for example, ^{125}I, ^{131}I, ^{213}Bi, ^{99}mTc, ^{111}In, ^{90}Y, or ^{32}P, such as for detection, imagining, diagnostic and therapeutic purposes.

Additional functional domains can also include indirect or regulated clearance domains. For example, an Aβ binding protein can comprise a biotin moiety and a streptavadin molecule such as galactosylated streptavadin can be used for clearance (Govindan et al. Cancer Biother Radiopharm. 2002 Jun;17(3):307-16).

3. Characterizing Aβ antibodiesand Aβ binding proteins

(a) Determination of Aβ Binding

Antibodies (including antibody fragments) and Aβ binding proteins described

herein can be assayed by any method known in the art for assessing binding to Aβ. Methods to assess binding include assays such as ELISA, western blotting, immunoprecipitation, two hybrid assays, phage display and others well known in the art. Binding assays can be used to ascertain if the prepared antibody or Aβ binding protein binds to Aβ. Binding assays can also be used to ascertain if the antibody or Aβ binding protein binds selectively to a particular Aβ. Aβ antibodies and binding proteins can be tested against a specific Aβ to determine which are preferentially bound. Peptides tested can include deletion variants of Aβ, including both N and C-terminal truncations of Aβ, as well as deletions within the central region of the Aβ peptide. Such peptides can be used to map the minimal amino acid sequences of Aβ recognized by an Aβ antibody or binding protein. For example, such binding assays can be used to demonstrate that the exemplary antibody A387 binds preferentiallyto Aβ42 with minimal or no binding to other Aβ peptides such as Aβ1-40 and Aβ1-39.

Methods known in the art can also be used to ascertain the relative binding affinity and avidity of the antibodies and Aβ binding proteins for Aβ and/or various forms of Aβ. For example, Aβ antibodies and binding proteins can be tested using binding assays such as ELISA, dot blots and immunoprecipitation with Aβ in soluble form, aggregates, low molecular weight oligomers, in plaques and neurofibrillary tangles. Such assays can be performed with isolated Aβ peptides or with samples taken from cells and tissues such as those of cell lines, animal models and subjects. Aβ can be solubilized and/or aggregated using *in vitro* methods such as sonication, and fibril growth in vitro (O'Nuallain et al., (2002) PNAS 99(3):1485-1490). Additionally, chemical reagents, such as metal chelators, can be used to generate low molecular weight forms of Aβ and then used to assays to assess the reactivity of an Aβ binding protein or Aβ antibody for the low molecular weight forms of Aβ. Assays can also be used to assess binding to specific molecular weight forms of Aβ such as monomers and low molecular weight oligomers or high molecular weight oligomers and aggregates. For example gel filtration and native gels can be used to assess the relative molecular weight or size of Aβ recognized by an Aβ antibody or Aβ binding protein. Western blotting and immunoprecipitation can also be used to assess selectivity of Aβ binding proteins and

antibodies for a particular Aβ. For example, as described in Example 9, Aβ can be treated with the metal chelator bathocuprione (BC) and then reacted with an Aβ antibody or Aβ binding protein in subsequent immunoassays. Such assays can be used to screen Aβ antibodies and Aβ binding proteins to isolate those specific for binding Aβ and particular Aβ peptides in a specific form or which bind only to a particular Aβ in a specific form. In one embodiment, antibodies are isolated which bind only to Aβ in low molecular weight forms. In another embodiment, antibodies are isolated which bind to Aβ42 and preferentially bind Aβ42 in low molecular weight forms. An exemplary antibody which binds selectively to Aβ42 and to Aβ42 preferentially in low molecular weight forms is the antibody A387.

(b) Clearance properties

Aβ antibodies and Aβ binding proteins can be assessed for their rate of clearance from the circulation using *in vivo* pharmacokinetic assays and/or *in vitro* assays that sufficiently correlate with *in vivo* results. Such assays are well known in the art (see, for example, Shargel and Yu (1999) "Applied Biopharmaceutics and Pharmacokinetics," 4th ed., McGraw-Hill/Appleton & Lange). For example, suitable assays can assess the half-life of the binding protein or antibody, and/or of bound Aβ, in cell-culture medium or blood; the uptake of the binding protein or antibody, and/or of bound Aβ, by a cell, tissue or organ; the intracellular or extracellular accumulation of degradation products of the binding protein or antibody; and the like.

In one type of *in vivo* assay, a detectably labeled (e.g. radiolabeled) Aβ binding protein or antibody is administered to a subject, and the decreasing level of label in the circulation, or the increasing level of label in the urine or liver, is monitored to assess the rate of clearance of the Aβ binding protein or antibody from the circulation. In another type of *in vivo* assay, an unlabeled Aβ binding protein or antibody is injected to a subject, and at various times after dosing, plasma is collected. Various assays can then performed to determine the concentration of administered protein remaining in the circulation. For example, an ELISA assay can be performed, using suitable capture reagents (e.g. Aβ) and detection reagents (e.g. a labeled secondary antibody). Alternatively, a radioimmunoassay (RIA) can be performed, in which the plasma Aβ binding protein or

antibody competes for binding of radiolabeled Aβ binding protein or antibody to a suitable secondary reagent.

In one type of *in vitro* assay, the uptake of detectably labeled Aβ binding protein or antibody from the culture medium by cells having receptors for the clearance domain is assessed. For example, if the clearance domain is a ligand for an Fc receptor, the cells can be macrophages. If the clearance domain is a ligand for LRP, because of the ubiquitous nature of LRP, the cells can be of essentially any tissue origin, such as hepatocytes and fibroblasts. After a suitable incubation period, cells are washed and the amount of intracellular label measured.

(c) Purification

Aβ antibody and Aβ binding protein purification may be carried out using standard protein purification techniques. Exemplary methods include ion exchange chromatography, HPLC, and affinity chromatography. Affinity chromatography using Protein A or Protein G. can be used to purify Aβ antibodies and Aβ binding proteins with antibody scaffolds. Affinity chromatography with Aβ peptides can be used to purify proteins which bind Aβ. Aβ antibodies and binding proteins can be generated with purification tags, such as a His_6 tag for metal binding, to facilitate purification. Such tags can be designed to be cleaved after the affinity purification step to produce purified Aβ antibodies and binding proteins. Purification can be assessed by standard methods known in the art such as electrophoresis and staining and mass spectrometry.

4. Expression of Aβ binding proteins

Numerous techniques are known in the art for the design of constructs to express Aβ binding proteins including Aβ antibodies and/or portions thereof. Expression constructs can be used for expression, for example, *in vitro* or *in vivo*, in cells, extracts, tissues or whole organisms. Such constructs are useful for assessing properties of Aβ binding proteins. Additionally, expression constructs are useful in the production of cell lines and transgenic organisms expressing Aβ binding proteins, including those used in screening methods described herein and known in the art.

a. Vectors and Constructs

A vector will generally contain elements useful for cloning and/or expression of

inserted nucleic acid molecules, such as an origin of replication compatible with the intended host cells; promoter, enhancer and/or other regulatory sequences, which can provide for constitutive, inducible or cell type-specific RNA transcription; transcription termination and RNA processing signals, such as a polyadenylation signal; one or more selectable markers compatible with the intended host cells (e.g. a neomycin or hygromycin resistance gene, useful for selecting stable or transient transfectants in mammalian cells, or an ampicillin or tetracycline resistance gene, useful for selecting transformants in prokaryotic cells); and versatile multiple cloning sites for inserting nucleic acid molecules of interest. The choice of particular elements to include in a vector will depend on factors such as the intended host cells, the insert size, whether expression of the inserted sequence is desired, the desired copy number of the vector, the desired selection system, and the like. Vectors suitable for use in cloning and expression applications include, for example, viral vectors such as a bacteriophage, adenovirus, adeno-associated virus, herpes simplex virus, vaccinia virus, baculovirus and retrovirus; cosmids or *Escherichia coli*-derived , *Bacillus subtilis*-derived and yeast-derived plasmids; bacterial artificial chromosome vectors (BACs) and yeast artificial chromosome vectors (YACs). Such vectors and their uses are well known in the art.

Nucleotide sequences that can be used to express proteins generally contain one or more transcriptional regulatory sequences (*e.g.* promoters, enhancers, terminators and the like) in operative association with the expressed sequence (e.g. an Aβ binding protein or portion thereof). Promoters for gene expression regulation include, for example, promoters for genes derived from viruses (*e.g.*, cytomegalovirus (CMV), Moloney murine leukemia virus (MMLV), JC virus, rous sarcoma virus (RSV), simian virus SV40, mouse mammary tumor virus (MMTV), *etc.*), promoters for prokaryotic expression such as T3 and T7 promoters, and promoters for genes derived from various mammals (*e.g.*, humans, rabbits, dogs, cats, guinea pigs, hamsters, rats, mice *etc.*) and birds (*e.g.*, chickens *etc.*) (*e.g.*, genes for albumin, insulin II, erythropoietin, endothelin, osteocalcin, muscular creatine kinase, platelet-derived growth factor beta, keratins K1, K10 and K14, collagen types I and II, atrial natriuretic factor, dopamine beta-hydroxylase, endothelial receptor tyrosine kinase (generally abbreviated Tie2), sodium-

potassium adenosine triphosphorylase (generally abbreviated Na,K-ATPase), neurofilament light chain, metallothioneins I and IIA, metalloproteinase I tissue inhibitor, MHC class I antigen (generally abbreviated H-2L), smooth muscle alpha actin, polypeptide chain elongation factor 1 alpha (EF-1 alpha), beta actin, alpha and beta myosin heavy chains, myosin light chains 1 and 2, myelin base protein, serum amyloid component, myoglobin, renin *etc.*). Inducible promoters such as chemically inducible promoters, for example, regulated by tetracycline, or steroids such as ecdysone, estrogen, or progesterone and others known in the art, may be used for expression.

The above-mentioned vectors can have a sequence for terminating the transcription of the desired messenger RNA in the transgenic animal (generally referred to as terminator); for example, gene expression can be manipulated using a sequence with such function contained in various genes derived from viruses, mammals and birds. The simian virus SV40 terminator and other known terminators known in the are commonly used. Additionally, for the purpose of increasing the expression of the desired gene, various other elements may be included: *e.g.*, the splicing signal and enhancer region of each gene, a portion of the intron of a eukaryotic organism gene may be ligated 5' upstream of the promoter region, or between the promoter region and the translational region, or 3' downstream of the translational region as desired.

Aβ binding proteins can be expressed as a single expression construct or may be expressed as multiple expression constructs. For example, an Aβ antibody comprised of a heavy and light chain can be produced by constructing an expression construct for heavy chain expression and a second expression construct for light chain expression. The two expression constructs may be contained on the same vector or on two separate vectors. They can be integrated together into a host cell or organism or alternatively integrated at different locations.

b. Cell culture production

Aβ binding proteins including Aβ antibodies and fragments thereof can be expressed in cell culture as a means of producing them for use in diagnostics, research or treatment. Expression in cell culture can also be used as the basis for characterizing and testing Aβ binding proteins and for further screening assays to identify molecules which

modulate or alter the interaction between Aβ binding proteins and Aβ.

Nucleic acid molecules can be introduced into host cells by various well-known transfection methods, including electroporation, infection, calcium phosphate co-precipitation, protoplast or spheroplast fusion, lipofection, micro-injection, and DEAE-dextran-mediated transfection (*e.g.* Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, N.Y. (1989); Ausubel et al., supra, (1999), Keown *et al.* (1990) *Methods in Enzymology* 185:527-537). Host cells can be maintained and propagated by methods known in the art (*e.g.* Freshney, R. I. (2000) "Culture of Animal Cells: A Manual of Basic Technique," 4th ed., Wiley-Liss).

Any cell line known in the art to be suitable for protein and/or antibody production can be used to produce Aβ binding proteins. Suitable host cells include human and other mammalian cells, including primary cells and cell lines. Exemplary host cells include mammalian primary cells (*e.g.* cells from any tissue of human, rabbit, dog, cat, guinea pigs, hamsters, rats, mice, etc.); embryonic stem cells, fertilized eggs and embryos; myeloma cells, cells contained in, or obtained from, transgenic animals; established mammalian cell lines, such as SY5Y, RBL, COS, CHO, HeLa, NIH3T3, HEK 293, BHKBI and Ltk$^-$ cells, mouse monocyte macrophage P388D1, J774A-1 and PC12 cells (available from ATCC, Manassas, VA); amphibian cells, such as *Xenopus* embryos and oocytes; avian cells; and other vertebrate cells. Exemplary host cells also include insect cells (e.g. *Drosophila*), yeast cells (*e.g. S. cerevisiae, S. pombe, Candida tropicalis, Hansenula polymorph* or *Pichia pastoris*), plant cells and bacterial cells (*e.g. E. coli*).

In some cases it may be desirable to modify the expressed proteins. In vitro can be used to accomplish modifications such as glycosylation, for example galactosylation and sialylation (Raju et al. Biochemistry. 2001 Jul 31;40(30):8868-76). Alternatively, in vivo modification can be accomplished by expression in cell lines which carry out such modifications or by the engineering of cell lines to provide the appropriate modifications (Choi, et al. Proc. Natl Acad Sci U S A. 2003 Apr 29;100(9):5022-7. Epub 2003 Apr 17).

Cell lines for or characterizing and testing Aβ binding proteins and for further

screening assays typically include cell lines that produce Aβ, for example primary cell cultures, typically neuronal cell cultures. Totipotent, pluripotent, or other cells that are not terminally differentiated can be induced to express neuronal characteristies including the production of Aβ peptides. Exemplary non-terminally differentiated cells include embryonic stem cells, adult stem cells, mesenchymal stem cells, bone marrow stem cells, adipose tissue stem cells, and neuronal stem cells. Additionally, cells can be engineered to express forms of Aβ of fragments thereof. Examples of such cell cultures, methods for induction of Aβ production, harvesting and culturing are described herein. Aβ binding proteins including Aβ antibodies can be added exogenously to cells expressing Aβ or expression of the Aβ binding proteins can be engineered within the same cell.

Nucleic acid encoding Aβ binding protein and Aβ antibody or portion thereof may be stably incorporated into cells or may be transiently expressed using methods known in the art. Stably transfected cells may be prepared by transfecting cells with an expression vector having a selectable marker gene (such as, for example, the gene for thymidine kinase, dihydrofolate reductase, neomycin resistance, and the like), and growing the transfected cells under conditions selective for cells expressing the marker gene. Transient expression may use similar methods without selectable markers or may use viral expression such as baculovirus, vaccinia virus, adenovirus and other transient systems known in the art.

Heterologous nucleic acid may be maintained in the cell as an episomal element or may be integrated into chromosomal DNA of the cell. The resulting recombinant cells may then be cultured or subcultured (or passaged, in the case of mammalian cells) from such a culture or a subculture thereof. Methods for transfection, injection and culturing recombinant cells are known to the skilled artisan. Expression of an Aβ binding protein mRNA or protein in cells can be assessed by methods known in the art such as Northern blotting, RT-PCR, Taqman, Western Blotting, ELISA, enzymatic function of an Aβ binding protein, and binding or interaction properties of an Aβ binding protein. Methods for protein expression and purification are known in the art (see, for example, Sambrook et al. (1989) MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. Cold Spring Harbor Laboratory Press; Ausubel et al. (1995) CURRENT

PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Co., NY; Rosenberg, I.M. (1996) "Protein Analysis and Purification: Benchtop Techniques" *Springer Verlag*; and Scopes, R.K. (1994) "Protein Purification: Principles and Practice" *Springer Verlag*.)

Biological compositions can be derived from cell lines such as but are not limited to, purified or partially purified enzyme preparations, conditioned medium from cultured cells, cellular extracts and cell lysates. Such compositions can be generated using methods described herein and/or known in the art for use in characterizing Aβ binding proteins and for further screening assays.

c. Transgenic Animals

Also provided herein are methods of producing transgenic animals by introducing nucleic acid encoding an Aβ binding protein into a cell and allowing the cell to develop into a transgenic animal. The cell may be any cell that may be used in the generation of a transgenic animal. Such cells are known to those of skill in the art of transgenic animal production. For example, the cell may be an embryo, zygote, oocyte, fertilized oocyte or embryonic stem cell, such as, for example, a mouse embryonic stem cell. Numerous techniques for introduction of exogenous nucleic acids into cells that will be allowed to develop into transgenic animals are also known to those of skill in the art. Such techniques include, but are not limited to, pronuclear microinjection (see, *e.g.*, U.S. Patent No. 4,873,191), retrovirus-mediated gene transfer into germ lines [see, *e.g.*, Van der Putten *et al.* (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*:6148-6152], gene targeting into embryonic stem cells [see, *e.g.*, Thompson *et al.* (1989) *Cell 56*:313-321], electroporation of embryos [see, *e.g.*, Lo (1983) *Mol. Cell. Biol. 3*:1803-1814], and sperm-mediated gene transfer [see, *e.g.*, Lavitrano *et al.* (1989) *Cell 57*:717-723] [for a review of such techniques, see Gordon (1989) *Int. Rev. Cytol. 115*:171-229]. A cell into which exogenous nucleic acid has been transferred may be introduced into a recipient female animal for development into a transgenic animal containing the exogenous nucleic acid.

Methods for making transgenic animals using a variety of transgenes have been described [see, *e.g.*, Wagner *et al.* (1981) *Proc. Nat. Acad. Sc. U.S.A. 78*:5016; Stewart *et al.* (1982) *Science 217*:1046; Constantini *et al.* (1981) *Nature 294*:92; Lacy *et al.* (1983)

Cell 34:343; McKnight *et al.* (1983) *Cell 34*:335; Brinstar *et al.* (1983) *Nature 306*:332; Palmiter *et al.* (1982) *Nature 300*:611; Palmiter *et al.* (1982) *Cell 29*:701, and Palmiter *et al.* (1983) *Science 222*:809; Ono *et al.* (2001) *Reproduction 122*:731-736; Reggio *et al.* (2001) *Biol. Reprod. 65*:1528-1533; Park *et al.* (2001) *Animal Reprod. Sci. 68*:111-120; Zakhartchenko *et al.* (2001) *Mol. Reprod. Dev. 60*:362-369; Arat *et al.* (2001) *Mol. Reprod. Dev. 60*:20-26; Koo *et al.* (2001) *Mol. Reprod. Dev. 58*:15-20; Polejaeva and Campbell (2000) *Theriogenology 53*:117-126]. Such methods are also described in U.S. Patent Nos. 6,175,057; 6,180,849 and 6,133,502, 6,271,436, 6,258,998, 6,103,523, 6,252,133.

d. *In vitro* and Synthetic systems

Aβ antibodies and Aβ binding proteins and fragments thereof can be produced *in vitro* in cell-free systems (Makeyev *et al.* (1999) *FEBS let.* 444:177-180). Such systems can be useful for rapid screening of constructs and mutants to ascertain function and binding specificity. For example, expressible antibodies and binding proteins can be constructed using PCR techniques to join a T7 or other known RNA polymerase tag onto the nucleotide sequence encoding the polypeptide. In vitro transcription and translation can then be used to express the polypeptides for use in binding or other assays. Single antibodies or binding proteins or libraries of such polypeptides can be produced by such methods.

Synthetic means can also be used to produce Aβ antibodies and Aβ binding proteins. For example, regions of Aβ antibodies and Aβ binding proteins can be synthesized in vitro and joined to scaffold molecules. Peptides of one or more CDRs of an Aβ antibody can be synthesized and tested for reactivity with Aβ.

J. Treatment of Disease and Disorders with Aβ binding proteins

Methods are provided herein for the use of Aβ binding proteins and Aβ antibodies in the treatment or prophylaxis of diseases involving or characterized by Aβ and/or specific Aβ forms.. Such diseases include, but are not limited to, diseases involving or associated with amyloidosis and neurodegenerative diseases. One example of such a disease is Alzheimer's disease.

Genetic and biochemical evidence indicates that accumulation of Aβ is involved in the pathogenesis of Alzheimer's and further that specific forms of Aβ, such as accumulation into oligomers, aggregates and plaques, participates in the pathogenesis of the disease. Immunization with Aβ peptides as well as passive immunization with Aβ antibodies has been shown to modulate both Aβ levels and related pathogenic and behavioral effects (Holtzman et al. (2002) Adv. Drud Delivery Rev. 54:1603-1613; Dodart et al., (2002) Nature Neurosci. 5(5):452-457; Bard et al., (2003) PNAS 100(4):2023-2028; WO00/72880). The methods are suitable for the treatment or prevention of disease because they are designed to selectively modulate Aβ levels. Methods herein are also provided to modulate the level of a particular Aβ, such as Aβ42.

Methods herein can include a step of administering an Aβ binding protein or Aβ antibody to a subject having such a disease or disorder or predisposed to such a disease or disorder. In one embodiment of the methods, the Aβ binding protein or Aβ antibody being administered is one that modulates the level of one or more Aβ peptides. In one embodiment, Aβ42 levels are modulated. The level of Aβ42 can be modulated to a greater extent than the level of one or more other Aβ peptides, in particular, Aβ40, such that the level of Aβ42 is modulated, or without substantially altering the level of one or more other Aβ peptides, in particular Aβ40. In a particular embodiment, Aβ42 levels are reduced.

In one embodiment, an Aβ binding protein or Aβ antibody being administered is one that preferentially binds a specific form of Aβ such as Aβ in low molecular weight forms. In one aspect of the embodiment, the Aβ binding protein or Aβ antibody is specifically reactive with a specific Aβ, in particular Aβ42, and also preferentially binds low molecular weight forms of Aβ42. In a particular embodiment, the A387 antibody or a fragment thereof is administered. In another embodiment, an Aβ binding protein which retains the binding specificity of the Aβ antibody for low molecular weight forms of Aβ42 is administered. For example, a humanized antibody that preferentially binds low molecular weight forms of Aβ42 is administered. In one embodiment, an antibody containing the sequence of SEQ ID NO:12 and/or SEQ ID NO:14, or portion

thereof, is administered.

In another embodiment, an Aβ binding protein or Aβ antibody being administered is one that recognizes the N-terminal region of Aβ. In a particular embodiment, the B436 antibody, or a fragment thereof is administered. In another embodiment, an Aβ binding protein which retains the binding specificity of the B436 antibody for N-terminal region of Aβ is administered. For example, a humanized antibody which retains the binding specificity of B436 is administered. In one embodiment, an antibody containing the sequence of SEQ ID NO:16 and/or SEQ ID NO:18, or portion thereof, is administered.

1. Predictive assays

Aβ binding assays such as those described herein and known in the art can be used to assess the reactivity of Aβ antibodies and Aβ binding proteins with Aβ. Determination of specificity, affinity, avidity as well as stability and clearance can assist in determining dosages and administration regimes. Assessment of the binding properties of Aβ antibodies and Aβ binding proteins can be ascertained for binding to specific forms of Aβ such as binding to Aβ in soluble or aggregate forms, binding of monomers, low molecular weight oligomers or high molecular weight aggregates. Assays such as those described herein for assessing binding to Aβ and specific Aβ peptides and forms of Aβ, and assays for clearance as well as additional methods known in the art can be used for assessing Aβ antibodies and binding proteins.

Animal models can also be used for the assessment of Aβ antibodies and Aβ binding proteins for the treatment of diseases and disorders associated with Aβ for example with altered Aβ levels, and/or altered ratios of one or more Aβ peptides and/or forms. In particular, non-human animals that have altered production, degradation and/or clearance of Aβ peptides or altered expression of APP can be used for such assays. Examples of such animals include transgenic animal models and animals, such as rodents, including mice and rats, cows, chickens, pigs, goats, sheep, monkeys, including gorillas, and other primates. Exemplary animal models include animals with the Swedish mutation of APP (Asp595-leu596), disclosedin US Patent Nos. 5,612,486 and 5,850,003, the transgenic mouse disclosed in US Patent No. 5,387,742, which expresses particular APP species that form β-amyloid protein deposits in the brain of the mouse,

and TASD41 transgenic mice, which express human APP751 cDNA containing the London (V717I) and Swedish (K670M/N671L) mutations under the control of the murine Thy-1 gene (Rockenstein *et al.* (2001) *J. Neurosci. Res.* 66:573-582). Additional transgenic animal models include those described in US Patent Nos. 5,811,633; 6,037,521; 6,184,435; 6,187,992; 6,211,428; and 6,340,783, transgenic mouse models Tg 2576; APPSWE mouse, K670N, M671L, and other models including APP(V717F), APP(K670N, M671L and V717F), PS-1 M146L, PS-1 M146V, APPSWE + PS A246E (reviewed by Emilien, *et al.*, (2000) *Arch. Neuro. 57*: 176-81).

Aβ antibodies and Aβ binding proteins can be administered, such as by injection, to animal models and the effects of such treatment assessed. For example, animals can be injected one or more times intraperitoneally, or by other suitable route, with an Aβ antibody or Aβ binding protein. Alternatively, transgenic expression can be used to produce an Aβ antibody or Aβ binding protein in an animal and the effects are assessed in the animal. For example, an Aβ antibody or Aβ binding protein can be expressed in a wildtype animal model and the animal is then assessed. An Aβ antibody or Aβ binding protein can also be expressed in a model animal for a disease or condition.

2. Administration of antibodies to subjects

Aβ antibodies and Aβ binding proteins can be administered to subjects for prophylactic and therapeutic uses. In prophylactic applications, a composition or medicament is administered to a subject at risk for a disease or condition such as Alzheimer's disease. In therapeutic treatments, a composition or medicament is administered to a subject suspected of or already suffering from a disease or condition, such as Alzheimer's disease. An amount of the composition or medicament is administered to achieve an effectiveness of treatment. As described herein, predictive assays such as in vitro and in vivo assays, including testing in animal models can be used to determine dosages and dosage regimes for treatment.

Dosages of Aβ antibodies and Aβ binding proteins for treatment will vary depending on conditions such as the means of administration, the target site, the species of subject and physiological state of the subject and the use of the treatment (e.g. prophylactic or therapeutic). Treatment dosages are optimized for safety and

effectiveness. Dosages range from 0.0001 to 100 mg/ kg of subject body weight. Typically, dosages are 0.01 to 10 mg/kg. In some cases, more than a single dose of the composition or medicament is necessary to achieve an effectiveness of treatment. For example, dosages can be daily, weekly, monthly or yearly. Dosages and dosage regimes can be determined empirically for example, by measuring the levels of Aβ, specific Aβ peptides and/or forms, and achieving a desired level of such in the subject by administering an Aβ antibody or Aβ binding protein to maintain that level. The dosages and dosage regimes can also depend on the stability of an Aβ antibody or Aβ binding protein. Stability of an Aβ binding protein or antibody can be determined by measuring levels of the protein or antibody in *in vitro* assays, cell based assays, in animal models and in a subject. For example, an amount of an Aβ antibody or protein can be administered to a subject and subsequent samples, such as blood, plasma or cerebral spinal fluid samples, taken from the subject over time to assess the amount remaining in the subject. In some cases an Aβ antibody or an Aβ binding protein with a detectable moiety such as a radiolabel, may be used to facilitate measurements.

Aβ antibodies and Aβ binding proteins can be administered by parenteral, topical, intravenous, oral, subcutaneous, interarterial, intracranial, intraperitoneal, intranasal and intramuscular means. Aβ antibodies and Aβ binding proteins can be administered to a particular organ or tissue, for example, by injecting directly into the organ or tissue. For example, Aβ antibodies and Aβ binding proteins can be injected directly in the cranium, into a muscle and directly into the bloodstream. For administration, Aβ antibodies and Aβ binding proteins can be formulated as a solution or suspension in a physiological diluent such as sterile water, saline, glycerol, oil or ethanol. Formulations can also be prepared as liposomes or micelles, microparticles and in formulation for sustained release. Formulations can also include surfactants, emulsifying agents, wetting agents, and pH buffering substances.

Aβ antibodies and Aβ binding proteins can also be administered in combination with other treatments, for example in combination another treatment for the disease or condition. For example, an Aβ antibody can be administered along with an agent that modulates the processing or levels of APP for treatment of Alzheimer's disease.

3. Assessment of Treatment

Methods for assessing treatment can be biochemical, physiological and/or can involve assessments of behaviors or phenotypes associated with a particular condition or disease. The effectiveness of treatment can include the effectiveness of a treatment to ameliorate symptoms such as by decreasing the severity, delaying the onset, delaying the recurrence, or decreasing the number of recurrences of symptoms or by delaying the progression of a disease or condition. Effectiveness of treatment also can include the effectiveness of a treatment to prevent a disease or condition, prevent the onset of symptoms of disease or condition. The effectiveness of ameliorating or preventing symptoms and/or the occurrence of a condition or disease can be assessed in animals, animal models and/or in subjects.

(a) Biochemical and Physiological Phenotypes

Levels and forms of Aβ can be observed after to treatment to ascertain changes in the levels of Aβ, such as levels of all Aβ peptides, levels of particular Aβ peptides, such as Aβ42, and changes in the form of Aβ, for example, the level of soluble Aβ and the level in plaques.

Aβ can be assessed in plasma for example after treatment and obtaining blood at sacrifice from animals by cardiac puncture. Blood is then centrifuged to obtain plasma which can then be tested for Aβ levels and forms by assays such as described herein or known in the art. For example, Aβ levels can be assed in an ELISA assay with Aβ antibodies. Additionally, the plasma can be tested for the level of treatment agent. For example, Aβ antibodies and/or Aβ binding proteins present in the sample can be detected by biochemical and/or immunological means. Levels and forms of Aβ can also be assessed in cerebrospinal fluid in a similar manner. Aβ can also be assessed in tissues such as the brain for example, by obtaining brain tissue from each animal at sacrifice. As described in the Examples herein, homogenates of brain sections can be analyzed for Aβ levels by ELISA or by other assays described herein or known in the art to assess Aβ levels and forms. Additional dissection into cortex, hippocampus and cerebellar regions before homogenization can be used to further localize Aβ.

Histopathology can also be used to assess treatment .For example as described in

the Examples, brain sections can be assayed for the abundance of amyloid plaques in treated and control animals. *In situ* analysis with antibody staining can also be used to ascertain levels of Aβ and Aβ forms, for example by using Aβ antibodies which recognize Aβ and/or specific Aβ forms (Dodart *et al.* (2002) *Nature Neurosci.* 5(5): 452-257). An Aβ antibody or Aβ binding protein with a detectable moiety can be used to detect the presence, level, stability and/or localization of the administered Aβ antibody or Aβ binding protein. For example, an initial dose of an Aβ antibody or Aβ binding protein with a detectable moiety can be administered and the level, stability and/or localization assessed to determine further dosing in the same animal or subject or to assist in predicting the dosage for additional animals or subjects to be treated.

(b) Behavioral Phenotypes

Behavioral phenotypes specific for an Aβ-associated condition or disease can be measured to ascertain the effect of treatment. For example, an assessment of Alzheimer's disease (AD) phenotype can refer to any visible, detectable or otherwise measurable symptom or property of an individual diagnosed with AD. Such properties include, but are not limited to, dementia, aphasia (language problems), apraxia (complex movement problems), agnosia (problems in identifying objects), progressive memory impairment, disordered cognitive function, altered behavior, including paranoia, delusions and loss of social appropriateness, progressive decline in language function, slowing of motor functions such as gait and coordination in later stages of AD, amyloid-containing plaques, which are foci of extracellular amyloid beta protein deposition, dystrophic neurites and associated axonal and dendritic injury, microglia expressing surface antigens associated with activation (*e.g.*, CD45 and HLA-DR), diffuse ("preamyloid") plaques and neuronal cytoplasmic inclusions such as neurofibrillary tangles containing hyperphosphorylated tau protein or Lewy bodies (containing α-synuclein). Standardized clinical criteria for the diagnosis of AD have been established by NINCDS/ADRDA (National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association) (McKhann *et al.* (1984) *Neurology 34*:939-944). The clinical manifestations of AD as set forth in these criteria are included within the definition of AD phenotype. For example, dementia may be

established by clinical exam and documented by any of several neuropsychological tests, including the Mini Mental State Exam (MMSE) (Folstein and McHugh (1975) *J. Psychiatr. Res. 12*:196-198; Cockrell and Folstein (1988) *Psychopharm. Bull. 24*:689-692), the Blessed Test (Blessed *et al.* (1968) *Br. J. Psychiatry 114*:797-811) and the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-COG) Test (Rosen *et al.* (1984) *Am. J. Psychiatry 141*:1356-1364; Weyer *et al.* (1997) *Int. Psychogeriatr. 9*:123-138; and Ihl *et al.* (2000) *Neuropsychobiol. 4*:102-107).

Tests can be developed in suitable laboratory animals to assess the effects of a treatment. For example, in AD, AD model animal can be treated and assessed. In one example, an object recognition task can be used to assess treatment. The test is based on the animal's spontaneous tendency to explore a novel object more frequently than a familiar one (Ennaceur et al. (1988) Behav. Brain Res. 31:47-59; Dodart et al. (1997) Neuroreport 8:1173-1178). Briefly, an animal such as a mouse is tested in a first trial with an object (such as a marble) and then in a second trial with the first object plus a new object (such as a die). A recognition index is calculated based on the amount of time the animal spends with each object in the second trial when both objects are present and the distance traveled toward each object.

Another example of a phenotypic test for AD is the holeboard memory task. (Dodart *et al.* (2002) *Nature Neurosci.* 5(5): 452-257). The test measures the ability of an animal to remember which holes of a holeboard have been baited with food. A food pellet is placed a hole of the board and the animal is tested in several trials over consecutive days where the same hole is baited each time. A global measure of cognitive performance is calculated from the trials based on the average number of errors made by the animal each day (based on entering holes never baited, re-entering a baited hole and not entering a baited hole).

Tests such as the object recognition task, holeboard memory task and other phenotypic assays known in the art are generally done with several animals to gather an average value. Single animals or groups of animals can undergo one or more treatments with a test agent, an Aβ binding protein, Aβ antibody, or any combination thereof and then treatment can be assessed with a phenotypic test. Control animals which have not

undergone any treatments or which have undergone placebo treatments can be compared to assess the effectiveness of a particular treatment relative to no treatment or placebo controls.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

Production of Aβ42-selective antibody (A387)

A selective Aβ42 antibody was produced by designing a peptide with the following sequence C-MVGGVVIA, which represents the $A\beta_{35}$-42 region with N-terminal cysteine added for conjugation to ovalbumin. Swiss-Webster mice were immunized with 1 mg of the conjugated peptide followed by three boosts of 0.5 mg antigen every three weeks. Following a third boost, spleens of these mice were fused to mouse B-cells. Hybridoma cells were cloned and screened for Aβ42 selectivity by determining antibody titer to both the Aβ40 and Aβ42 peptide (AnaSpec, Inc. San Jose, CA) by ELISAs (as described below). Positive clones which had selective reactivity to the Aβ42 peptide were chosen. The cells were then injected intraperitoneally into SCID mice and ascites fluid was obtained and purified using Protein A. Titer of antibodies produced was determined by coating 50 μl of Aβ peptide (AnaSpec, Inc, San Jose, CA) in PBS (500 ng/ml) on CoStar 3590 microtiter 96-well plates. Wells were blocked with 200 μl of 3% BSA/PBS (Sigma, St. Louis, MO) and incubated with antibody for 1 hour at room temperature. Wells were washed three times with 200 μl of PBS/0.1% Tween-20 (Sigma, St. Louis, MO). After washing, wells were incubated with mouse:horseradish peroxidase (HRP) secondary antibody for 1 hour at room temperature. Wells were washed three times with 200 μl of PBS/0.1% Tween-20. 50 μl TMB (3,3',5,5'-tetramethylbenzidine) substrate was then added according to manufacturer's recommendations (KPL, Gaithersburg, MD) and incubated for 15 min. The reaction was stopped with 50 μl of 9.8% phosphoric acid (Milwaukee, WI) and the absorbance at 450 nm was quantitated by a Biorad® 96-well plate reader. One antibody, designated A387, was found to have >1000 fold specificity for Aβ42 versus Aβ40 with a very high titer as

determined in the above ELISA. Additionally, this antibody was shown to be specific for Aβ42 versus other AB peptides; AβB1-11, 1-28, 1-38, and 1-39 when tested in the above assay. Antibody A387 was subtyped and confirmed to be IgG2a kappa. This antibody was then used to develop an Aβ42 assay to quantitate Aβ42 peptide produced by cells.

EXAMPLE 2

Production of Aβ1-12 antibody (B436)

An antibody that recognizes the amino-terminal 1-12 amino-acid region on Aβ was produced and conjugated to alkaline phosphatase for use as a detection antibody in the Aβ42 sandwich ELISA. The Aβ1-12 antibody was produced by designing a peptide with the following sequence DAEFRHDSGYEV-C that represents the Aβ1-12 region with a C-terminal cysteine added for conjugation to ovalbumin. Swiss-Webster mice were immunized with 1 mg of the conjugated peptide followed by three boosts of 0.5 mg. Following a third boost, spleens of these mice were fused to mouse B-cells. Hybridoma cells were cloned and screened for Aβ reactivity. The cells were then injected intraperitoneally into SCID mice and ascites was obtained and purified using Protein A. One antibody, designated B436, was found to have high titer for both Aβ40 and Aβ42 peptides, this was a desired feature since this antibody should equally react to any Aβ peptide which contains the 1-12 amino-terminal portion of the peptide. This antibody was subtyped and confirmed to be IgG2a kappa and was further purified by affinity chromatography on an Aβ1-12:Sepharose column and then conjugated to alkaline phosphatase. This antibody was then used as the detection antibody in the development of the Aβ42 assay to quantitate Aβ42 peptide produced by cells.

EXAMPLE 3

Production of LRP polyclonal antibody (R9377) for detection of LRP C-terminal fragments

A polyclonal antibody that recognizes the C-terminal region on LRP designated R9377 was prepared to the carboxyl-terminal 13 amino acid peptide (C-GRGPEDEIGDPLA) of LRP which was conjugated to ovalbumin via an amino-terminal cysteine residue incorporated into the LRP peptide. Initially, rabbits were primed with Complete Freund's adjuvant then immunized 14 days later with 1 mg of conjugated

antigen and Incomplete Freund's adjuvant. Following this immunization, the rabbits received monthly boosts of antigen/Incomplete Adjuvant (0.5 mg). 14 days following the third boost, serum was collected and IgG was purified using Protein A:Sepharose. The purified antibody was used in the immunoblotting experiments described in Example 8.

EXAMPLE 4

Aβ42 (A387) and Aβ1-12 (B436) monoclonal antibody cDNA sequencing Protocol

(1) RNA Extraction

One confluent plate (approximately $1.5x10^7$ cells) each of A387 and B436 A-beta mAb cell lines was harvested, pelleted, washed in 1X PBS, quick-frozen, and stored at -80°C. Using the RNeasy Mini Kit (QIAGEN #74104) according to manufacturer's protocol, the cells were lysed, homogenized by vortexing, and total RNA was extracted from half of each lysate.

(2) cDNA Synthesis

First-strand cDNA synthesis was performed using the SuperScript First Strand cDNA Synthesis System for RT-PCR (Invitrogen #11904-018) with antisense primers specific for *Mus musculus* kappa light chain and IgG_{2a} heavy chain sequences (GenBank accession numbers D14630 and V00765, respectively). The antisense primer sequences are as follows: light chain, 5'-GGACGCCATTTTGTCGTTCACTGCCA-3' (Kappa_LCC; SEQUENCE ID NO: 22); heavy chain, 5'-TGTTGTTTTGGCTGAGGAGACGGTGA-3' (IgG2a_HCC; SEQUENCE ID NO. 23). Duplicate reactions containing 2.5 μg A387 or B436 total RNA were prepared with or without reverse transcriptase (+RT and -RT, respectively) according to the manufacturer's protocol.

(3) PCR

DNA encoding the A387 and B436 light and heavy chain variable regions were amplified by touchdown polymerase chain reaction using the Expand High Fidelity System (Roche #1732641), degenerate sense primers, and the Kappa_LCC and IgG2a_HCC antisense primers. The sense primers were designed using the sequence of 12-15 N-terminal residues from each heavy and light chain, previously obtained by N-

terminal amino acid sequencing performed according to standard procedures by the Protein Core Facility at the University of Nebraska on a fee for service basis. These sequences were back-translated using Vector NTI 7 software (Informax, Inc.), reducing the level of degeneracy by applying a human codon preference table. The sense primer sequences are as follows: A387 light chain 5'-GAYATYGTSCTSACNCAGWSBCCNGC-3' (A387_LCV1; SEQUENCE ID NO. 24) A387 heavy chain, 5'-GARGTYAAGYTBGTYGARTCYGGAGG-3' (A387_HCV1; SEQUENCE ID NO: 25); B436 light chain, 5'-GAYGTYYTBATGACYCARACYCCA-3' (B436_LCV1; SEQUENCE ID NO: 26)); and B436 heavy chain, 5'-GARGTYATGYTBGTYGARTCYGGAGG-3' (B436_HCV1; SEQUENCE ID NO. 27). Reaction mixtures were prepared according to the manufacturer's protocol for each A387 or B436 +RT and -RT reaction. Amplification was performed in a Perkin-Elmer 3700 thermocycler according to the following conditions: denaturation for 2 min at 94°C; 10 cycles of 15 sec at 94°C, 1 min at 70°C-0.5°C per cycle, 1 min at 72°C; 10 cycles of 15 sec at 94°C, 1 min at 65°C, 1 min at 72°C; 25 cycles of 15 sec at 94°C, 1 min at 65°C, 1 min +5 sec/cycle at 72°C; and a final extension for 7 min at 72°C.

(4) Cloning

PCR products were analyzed by gel electrophoresis on a 1% agarose gel. A major band of the approximate expected size (light chain: ~487 bp; heavy chain: ~408 bp) was observed in each +RT reaction. An additional approximately 300-bp band was observed in the B436 reaction. No products were detected in the corresponding -RT control reactions. The desired ~487-bp and ~408-bp bands were purified using the QIAquick Gel Extraction Kit (QIAGEN #28704) according to the manufacturer's protocol. The TOPO TA Cloning Kit (Invitrogen #K4600-01) was used to clone each product into vector pCR®II-TOPO and transform E. coli TOP10 cells, according to the manufacturer's protocol. PCR analysis of transformants using T7 and SP6 primers identified 9 putative A387 light chain and 12 each putative A387 heavy chain, B436 light chain and B436 heavy chain constructs. Plasmid DNA was prepared for each of these from bacterial cultures using the QIAprep Spin Plasmid Kit (QIAGEN #27106) according to the manufacturer's protocol.

(4) **Sequencing**

The cDNA inserts were sequenced with the ABI Prism BigDye Terminators v.3.0 Cycle Sequencing Kit (ABI #4390244) using approximately 250 ng of each plasmid and 1.6 μM each of standard T7 and SP6 primers. The manufacturer's protocol for 20 μl reactions was followed, except that the BigDye reagent was reduced to 2 μl and supplemented with 4 ul 5X Sequencing Buffer (ABI #4305603) per reaction. Reactions were purified using the CleanSEQ Kit (Agencourt #000136) according to the manufacturer's protocol then analyzed on an ABI 3700 sequencer. The results were evaluated using Sequencher software (Gene Codes Corp.). A387 light chain nucleotide sequences were obtained from seven independent clones. The identity of every nucleotide between the primer binding sites was confirmed by agreement between at least six of those sequences, with 99% of the sequence identical in all seven clones. Similarly, the identity of each nucleotide was confirmed in at least 10 of 11 A387 heavy chain clones, 11 of 12 B436 light chain clones, and 11 of 12 B436 heavy chain clones, with >99% of the sequences identical in all clones for each case. Some nucleotide sequence variability was seen in the N-terminal primer binding sites due to primer degeneracy. However, the amino acid sequences for these regions was previously determined by N-terminal amino-acid sequencing.

The nucleotide sequences obtained are provided in SEQ ID NO: 11 (A387 light chain variable region (nucleotides 1-285), J region (nucleotides 286-321) and N-terminal sequence of a constant region (nucleotides 322-478)), SEQ ID NO: 13 (A387 heavy chain variable region (nucleotides 1-291), DJ region (nucleotides 292-354) and N-terminal sequence of a constant region (nucleotides 355-366)), SEQ ID NO: 15 (B436 light chain variable region (nucleotides 1-300), J region (nucleotides 301-336) and N-terminal sequence of a constant region (nucleotides 336-493)), SEQ ID NO: 17 (B436 heavy chain variable region (nucleotides 1-294), DJ region (nucleotides 294-342) and N-terminal sequence of a constant region (nucleotides 342-354)). The nucleotide sequences (and encoded amino acid sequences) are also provided in SEQ ID NOs: 37 and 38 (A387 light chain nucleotide and amino acid sequences, respectively), 39 and 40 (A387 heavy chain nucleotide and amino acid sequences, respectively), 41 and 42 (B436 light ehain

nucleotide and amino acid sequences, respectively), 43 and 44 (B436 heavy chain nucleotide and amino acid sequences, respectively).

Results

Key: V-J regions are underlined. Regions determined by N-terminal amino acid sequencing are double-underlined. Regions not underlined are the N-terminal portions of the C regions.

A387 light (kappa) chain (SEQUENCE ID NO. 12)

1	DIVLTQSPAT	LSVSPGDSVS	LSCRASQSIS	NNLHWYQQKS	HESPRILIKY
51	ASQSIYGIPS	RFSGSGSGTE	FTLIVNSVGT	EDFGMYFCQQ	SHSWPLTFGT
101	GTKLELKRAD	AAPTVSIFPP	SSEQLTSGGA	SVVCFLNNFY	PRDINVKWKI
151	DGSERQNGV				

A387 heavy (IgG_{2a}) chain (SEQUENCE ID NO.14)

1	EVKLVESGGD	LVKPGGSLKL	ACAASGFTFS	NDAMSWVRQT	PEKRLEWVAS
51	ISSVGNTYYP	DSVKGRFTIS	RDNARNILYL	QMSSVRSEDT	AMYYCARGYG
101	VSPWFSYWGQ	GTLVTVSSAK	TT		

B436 light (kappa) chain (SEQUENCE ID NO. 16)

1	DVLMTQTPLS	LPVSLGDQAS	ISCRSSQNIV	HSSGNTYLEW	YLQKPGQSPK
51	LLIYKVSNRF	SGVPDRFSGS	GSGTDFTLKI	SRVEAEDLGI	YYCFQGSHVP
101	YTFGGGTKLE	IKRADAAPTV	SIFPPSSEQL	TSGGASVVCF	LNNFYPRDIN
151	VKWKIDGSER	QNGV			

B436 heavy (IgG_{2a}) chain (SEQUENCE ID NO. 18)

1	EVMLVESGGG	LVKPGGSLKL	SCVASGFTFS	RYTMSWVRQT	PAKRLEWVAT
51	INFGNGNTYY	PDSVKGRFTI	SRDNARNTLY	LQMSSLRSED	TAMYYCTSLN
101	WAYWGQGTLV	TVSSAKTT			

EXAMPLE 5

Aβ42 and Aβ40 Sandwich ELISAs

Sandwich ELISAs (Enzyme-Linked Immunosorbent Assays) have been developed for specific detection of Aβ42 and Aβ40 peptides. An anti-Aβ42 selective monoclonal antibody or anti-Aβ40 selective monoclonal antibody (prepared to Aβ30-40

peptide using the same protocol as described for Aβ42 antibody production) was coated on white microtiter 96-well plates (50 μl at ~5 μg/ml) in PBS, pH 7.4. Following overnight coating at 4 °C, wells were blocked with 200 μl of 3% BSA, Fraction V (Sigma, St. Louis, MO) and incubated with Aβ peptides for 1 hour at room temperature. Wells were washed three times with 200 μl of PBS/0.1% Tween-20. After washing, wells were incubated with anti-Aβ1-12 conjugated to alkaline phosphatase (~0.5 μg/ml) for 1 hour. Wells were washed three times with 200 μl of PBS/0.1% Tween-20 and CDP-Star chemiluminescence substrate (Tropix, Inc.) was added at 50 μl/well and incubated for 15 min. The luminescence was then quantified on an ABI luminometer. Results show a large linear range of 75-2000 pg/well, high dynamic range of 3-30 fold over background in linear range (signal:noise), low sensitivity limit <20 pg/well, and >1000-fold selectivity for Aβ42 over other Aβ peptides, making the assay highly amenable to high throughput screening.

EXAMPLE 6

AB42/Aβ40 high-throughput screening assay

A selective Aβ42/Aβ40 high throughput 384-well screen to identify compounds that do not affect Aβ40 levels has been developed. Due to the high sensitivity and selectivity of the Aβ42/Aβ40 ELISA, this assay was formatted for use in 384-well plates for high throughput screening for compounds that selectively decrease Aβ42 levels while not affecting Aβ40 levels.

Human neuroblastoma cells (SH-SY5Y) were obtained from ATCC (CRL-2266) and transfected with human APP_{751} in a pcDNA.1 vector containing a neomycin resistant site. Cells were selected with 400 μg/ml G418 (Gibco) and cloned by limiting dilution. Cells expressing the amyloid precursor protein (APP_{751}) were plated in 384-wells and allowed to adhere for 24 hours. The cells were treated with a dose-response of DAPT (a positive control inhibitor used to inhibit Aβ42 production) ranging from 1 nm to 1 μM for 18 hours. Supernatant was then removed and assayed in the Aβ42 ELISA. The ELISA was carried out by coating white microtiter 384-well plates with 25 μl of ~5 μg/ml solution of Aβ42 selective monoclonal antibody (A387) in PBS. Following overnight coating at 4 °C, wells were blocked with 50 μl of 3% BSA/PBS, Fraction V

(Sigma, St. Louis, MO) and incubated with cell supernatant for 1 hour at room temperature. Plates were washed three times with 50 μl of PBS/0.1% Tween-20. After washing, wells were incubated with 25 μl of anti-Aβ1-12 conjugated to alkaline phosphatase (~0.5 μg/ml) for 2 hours. Wells were washed three times with 50 μl of PCS/0.1% Tween-20 and 25 μl of CDP-Star chemiluminescence substrate (Tropix, Inc.) was added and incubated for 30 minutes at room temperature. Luminescence was quantified on an Analyst HT. The assay was repeated with a test library of compounds. Compound concentrations were ~30 μM. 1 μM DAPT was used as a positive control and DMSO vehicle alone (0.12%) was added as a negative control. The data showed acceptable signal to background (~7-10 fold) with the positive control wells clearly distinguishable from the vehicle controls. Data from the test library screen showed that the hit criteria of <50% of plate median (50% inhibition)) is outside the normal distribution of the data therefore, compounds showing >50% inhibition in this primary screen were chosen for further follow-up assays such as Aβ40 inhibition and cytotoxic assays. The % coefficient of variation range measured was 15-17%. Taken together, these data indicate that the assay (when performed in duplicate) has a >95% chance of identifying inhibitors.

Test compounds which show >50% inhibition for Aβ42 levels are then tested for their effects on Aβ40 levels using a similar assay except that the coat antibody is Aβ40-specific. Furthermore, compounds are assessed for cytotoxicity using Alamar Blue (Biosource, Camarillo, CA) according to manufacturer's recommendations. Briefly, 10% Alamar Blue is added to cells after incubation of compound for 18h and incubated for 4 hours at room temperature, after which fluorescence is read on a spectrophotomer. Compounds that showed >40% cytotoxicity were eliminated as hits. The screening methods have also been performed using CHO cells containing DNA that encodes human APP_{695} and human PS1. The screening methods may also be performed using mouse neuroblastoma (N2a) cells expressing APP. N2a cells can be transfected with DNA encoding APP as described in Example 8.

EXAMPLE 7

Analysis of Processing of LRP

Endogenous LRP protein of N2a cells expressing human wild-type and mutant PS1 was analyzed and compared. Notch and APP protein in the cells was also analyzed as a reference for PS1-dependent protein cleavage.

Stable recombinant N2a cells that had been transfected with DNA encoding wild-type human APP695 (see, *e.g.*, SEQ ID NO: 30 and GenBank Accession no. Y00264) and DNA encoding either wild-type human PS1 (see, *e.g.*, SEQ ID NO: 5) or mutant human PS1 were grown overnight to near 70% confluence in a 10-cm tissue-culture dish. Two mutant PS1 cell lines were used: Δ1,2 and D385A. The Δ1,2 cell line expresses defective (i.e., loss of function) PS1 proteins encoded by nucleic acid lacking exons 1 and 2 of the human PS1 gene. The D385A cell line contains nucleic acid coding for an alanine instead of an aspartic acid residue at amino acid 385 (see, *e.g.*, SEQ ID NO: 6 for amino acid sequence of a wild-type human PS1) which is essential to PS1 function.

The Δ1,2 cells and the D385A cells were also transiently transfected with 2 μg of DNA encoding an amino-terminal truncated form of human NOTCHΔE containing residues 1-26 (signal sequence; see, *e.g.*, SEQ ID NO: 31) and residues 1718-2195 (see, *e.g.*, SEQ ID NO: 31) with the methionine 1738 mutated to valine to prevent alternative translation initiation at that site. The DNA construct also contains nucleic acid sequence encoding a carboxy-terminal V5 antibody epitope which is comprised of the 14-amino acid sequence; Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr added to the carboxy-terminal end of the Notch amino acid sequence so that a V5 antibody could be used to detect the NotchΔE or the NICD. This construct encodes a ~55-60 kDa protein. Transfection was carried out using Quiagen's effectene reagent for 20 h. Cells were then plated at 1.2 x 10^6 cells/well in 6-well plates. After 28 h, cells were treated +/- DAPT (1 μM) and then lysed in 200 μM lysate buffer (10% 10X TBS, 0.05% Tween 20, 1% Triton X-100, and a protease inhibitor cocktail) after 19 h of treatment. Cells were centrifuged at 10,000 rpm for 5 min and the supernatant was removed. The supernatant of the lysates was then separated on 8% Tris-Glycine gels and proteins were transferred to nitrocellulose membrane. The membranes then were blocked for an hour with 10% nonfat dry milk and probed with the anti-V5 (1:2000) primary antibody (Invitrogen, San Diego) to detect accumulation of the Notch substrate, anti-LRP antibody R9377 (as

described in Example 3) to probe for LRP CTFs, and anti-APP antibody R8666 (a rabbit antibody prepared to the carboxy-terminal region; amino acids C-EVPTYKFFEQMQN conjugated to ovalbumin through the amino-terminal cysteine residue) to visualize APP CTFs. Bound antibody was detected using the ECL SuperSignal system (Pierce) after incubation with anti-rabbit horseradish peroxidase-coupled secondary antibodies (Sigma). Samples were assayed in duplicate.

In lysates of the wild-type PS1 cells, an approximately 20 kDa protein fragment was observed in the presence of the PS1 inhibitor DAPT. The fragment is one that is recognized and bound by the polyclonal antibody R9377 generated against a carboxyl-terminal peptide (the carboxyl-terminal 13 amino acids) of human LRP (C-GRGPEDEIGDPLA) and thus is one derived from a C-terminal portion of LRP. Accumulation of this fragment was not detected in lysates of wild-type PS1 cells not treated with DAPT. Because little to no protein is detected in DAPT-treated cell lysates by the R9377 antibody generated against a C-terminal peptide of LRP, but a peptide fragment is detected at significant levels by the antibody in lysates of DAPT-treated cells, it can be concluded that a PS1-dependent activity cleaves LRP in such a way as to eliminate the epitope sequence on LRP that is recognized by antibody R9377. These results are consistent with presenilin-dependent cleavage of LRP.

Similar results were obtained in the analyses of lysates of wild-type PS1 cells using antibodies reactive with APP and Notch, respectively. In lysates of wild-type PS1 cells treated with DAPT, two peptide fragments (~17 kDa and ~14 kDa representing the C99 and C83, β- and α-secretase cleavage products, respectively) were readily detected by the anti-APP antibody R8666. In lysates of cells that were not treated with DAPT, little to no protein was detected by the R8666 antibody. In lysates of wild-type PS1 cells treated with DAPT, one peptide fragment was detected by the anti-V5 antibody. Although this fragment was also detected in lysates of wild-type PS1 cells that were not treated with DAPT, the amount of the fragment detected in the lysates of the DAPT-treated cells was significantly greater than in the lysates of the untreated cells.

The results of the analyses of lysates of DAPT-treated and untreated wild-type PS1 cells using anti-APP and anti-Notch-V5 fusion protein antibodies are consistent with

inhibition and non-inhibition, respectively, of the PS1-dependent cleavage of these presenilin substrates (i.e., APP and Notch) at a site in a C-terminal portion of these proteins. Thus, the similar findings in the analysis of LRP protein in the cell lysates and the APP and Notch analyses supports the conclusion of a presenilin-dependent cleavage of LRP.

In the lysates of the DAPT-treated and untreated Δ1,2 mutant cell line, the ~20 kDa LRP CTF was equally evident at significant levels. Similarly, the same fragments were detected in the lysates of the DAPT-treated and untreated Δ1,2 mutant cells by the R8666 antibody, and the anti-Notch-V5 fusion protein antibody also detected the same fragment in lysates of the treated and untreated mutant cells. The same results were obtained in analyses of the lysates of the DAPT-treated and untreated mutant D385A cells with the antibodies for the detection of LRP, APP and Notch peptides. These results obtained with cells that do not express functional PS1 provide confirmation that the results observed with DAPT-treated and untreated wild-type PS1 cell lysates are due to the inhibition and non-inhibition of a PS1-dependent activity.

Furthermore, a comparison of the very minimal levels of the peptide fragments detected in immunoassays of lysates of wild-type PS1 cells that were not treated with DAPT with the significant levels of the peptide fragments detected in lysates of D385A mutant cells, indicated an approximate 40-60% loss of PS1 activity in the mutant cells relative to wild-type PS1 cells. Because accumulation of the ~20 kDa LRP fragment in the presence of DAPT and in PS1 mutant cell lines parallels the accumulation of the APP and Notch fragments, these results indicate that LRP undergoes a PS1-dependent cleavage.

EXAMPLE 8

Presenilin/γ-secretase Assays

N2a mouse neuroblastoma cells (ATCC, Rockville, MD) transfected with APP_{WT} (Acc. No. Y00264) were incubated with DAPT (1 μM or 1 mM) or vehicle control (DMSO) for 24 hours. Lysates were then prepared by first washing the cell layer three times with isotonic PBS. To each 96-well, 50 ml of lysate buffer (TBS, 1% Triton X-100, 5 mM EDTA, 0.2% Tween-20, 10 μM leupeptin, 1 mM PMSF) was added and cells

were removed by agitating with a pipet tip. Cell lysates were spun for 5 min at 10,000 rpm in a microfuge and the supernatant was collected.

The lysates were then separated on 4-20% Novex gels and probed by immunoblotting with the anti-LRP polyclonal antibody (R9377). Results showed accumulation of a 20 kDa protein in lysates of cells that had been treated with DAPT. This band represented a carboxyl-terminal fragment of LRP. This accumulation of LRP CTFs paralleled the accumulation of APP CTFs, a finding that demonstrates that LRP is a distinct presenilin substrate and can be used to quantitate presenilin activity. The LRP assay can be used to profile test compounds that modulate Aβ levels, and, in particular Aβ42 levels (such as can be identified in the high-throughput assay; see EXAMPLE 6), with respect to possible effects on presenilin activity. In one aspect, compounds that are identified as agents that reduce Aβ42 levels (*e.g.*, by $\geq$50% at, *e.g.*, 30 μM; see EXAMPLE 6) are tested for any effects on presenilin activity in the LRP assay in order to identify Aβ42-reducing compounds that have minimal to no inhibitory activity with respect to presenilin-dependent LRP processing activity. Compounds were chosen that had <20% increase (at the highest tested concentration of 30 μM) of LRP-CTFs as compared to the DAPT positive control.

EXAMPLE 9

Characterization of binding properties of Aβ antibodies

(1) Assessment of binding to different forms of Aβ

(a) Non-reducing gel electrophoresis and immunoblotting

Methods. Aβ40 or Aβ42 peptide standards (Bachem) (250 ng in 10 μl) were mixed with 10 μl native sample buffer (Invitrogen). Samples were run on 18% Novex 10-well gels at constant voltage (150 V) using native sample buffer (Invitrogen). Novex rainbow standards (250 kd to 4 kd) were used as molecular weight controls. When the dye front reached the bottom of the gel, proteins were transferred to 0.45 micron PVDF filters (pre-wetted in methanol) at 100 mA constant current in 1X CAPS buffer (10 mM

CAPS), 10% methanol, pH 11.0 for 90 min. Filters were blocked with TBS, 10% dry milk, pH 7.4 for 60 min at room temperature. Filters were then incubated overnight at 4°C in a solution of TBS, 3% dry milk, 0.1% Tween-20 containing primary antibody (1-5 μg/ml of A387 or B436 conjugated to biotin), followed by three five-minute washes in TBS containing 0.1% Tween-20. After washing, filters were incubated in anti-biotin peroxidase-conjugated secondary antibody (Sigma; 1:2000 in TBS, 3% dry milk, 0.1% Tween-20) for 2 h at room temperature, and washed six times for five minutes each with TBS, 0.1% Tween-20. Signals were detected with the Chemiluminescence Supersignal ECL system (Pierce).

Results. By non-denaturing gel electrophoresis and immunoblotting as described above, Aβ42 peptides run at positions consistent with various forms, including insoluble fibrils (near top of the gel), high molecular weight oligomers (> ~100 kd) and low molecular weight oligomers such as pentamers (~20 kd) and dimers (~10 kd). Aβ40 peptides run at positions consistent with insoluble fibrillar and low molecular weight oligomeric forms. B436 antibody was shown to detect all forms of both Aβ40 and Aβ42 peptides. As expected, A387 did not recognize any forms of Aβ40. A387 antibody did not significantly recognize either Aβ42 fibrils or high molecular weight oligomers, and instead primarily recognized Aβ42 low molecular weight oligomers.

(b) ELISA assays in presence and absence of bathocuprine

Methods. Dynex Microfluor-2 White Flat-bottom 96-well plates were coated overnight with 2-10 μg/well of A387 antibody. Aβ42 peptide standard (Bachem) was added at concentrations ranging from 5000 pg/well to 0.08 pg/well at half log intervals, in either DMEM complete medium or DMEM complete medium containing 2 mM of the metal chelator bathocuprine, and plates incubated at room temperature for 2 h. After washing in PBS/0.1% Tween-20, alkaline phosphatase-labeled B436 antibody (~0.5 μg/ml) in 1% BSA/TBS/0.1% Tween-20 was added, and plates incubated at room temperature for 2 h. After washing, CDP-Star-Sapphire Luminescence Substrate

(Applied Biosystems) was added and the plates incubated for 5-15 min in the dark. Signal was then detected using an ABI TR717 Luminometer.

Results. Bathocuprine has been shown to solubilize Aβ aggregates into low molecular weight oligomers (Cherny et al. (1999) *J. Biol. Chem.* 274:23223-23228). In the presence of bathocuprine, A387 bound more Aβ42 peptide than in the absence of bathocuprine. These results are consistent with A387 recognizing lower molecular weight oligomers of Aβ42 peptide.

(2) Assessment of antibody binding to Aβ in plasma

Methods. Mouse blood was obtained by cardiac puncture at sacrifice. Briefly, mice were sedated by standard anesthesia. Upon sedation, each mouse was placed in dorsal recumbence and a 26-gauge needle attached to a heparinized 1 cc syringe inserted into the thorax through the diaphragm to an approximate depth of 2 cm. Light suction was applied to the needle and placement in the cardiac (ventricular) chamber of the mouse confirmed by blood flow to the syringe chamber. Blood was aspirated until flow ceased. To obtain plasma, blood samples from each mouse were spun at 3,000 RPM for 10 minutes, and the supernatant collected. Plasma was then frozen until analyzed.

For the immunoprecipitation assay, mouse plasma was centrifuged at low speed for 5 min to remove precipitated material, and the supernatant diluted in PBS 1:2. Human Aβ40 and Aβ42 peptide standards (Bachem) were added at 300 ng/ml to the diluted plasma, and the samples incubated with 1 ml of Sepharose beads for 1 h at 4°C. Beads were precipitated, and samples divided into 1 ml aliquots. To 1 ml of sample, biotin-labeled B436 or A387 antibodies (~10 μg/ml) were added, together with 40 μl of Streptavidin:Sepharose beads (Pierce), and the samples rocked overnight at 4°C. Samples were spun to pellet the beads, which were washed twice with 1 ml PBS-0.1% Tween-20. 30 μl of NuPAGE sample buffer (Invitrogen) was added, samples were boiled for 3 min, and supernatants loaded onto a 10% Bis-Tris NuPAGE gel (Invitrogen) at 125V. Molecular weight standards from 185 kDa to 3 kDa were also run. After

electrophoresis, proteins were transferred to PVDF filters at 100 mA for 90 min. Filters were then blocked in TBS-Tween containing 5% non-fat dry milk for 2 h. Blocked filters were incubated overnight with biotin-labeled 6E10 antibody, which recognizes the N-terminus of Aβ (1:500; Signet Laboratories), and then with HRP-labeled anti-HRP for 1 h (1:2000; Sigma). Signal was detected following incubation with Super Signal (Pierce Chemical Co.) for 1 min.

Results. Both A387 and B436 antibodies were able to immunoprecipitate human Aβ42 spiked into mouse plasma. The detected Aβ42 had an apparent molecular weight consistent with monomeric form of the peptide.

(3) Assessment of binding to Aβ in brain

Methods. The animals used in these experiments were either C57 mice, or Tg2576 mice of three or six months of age. Tg2576 mice express human APP695 with the Swedish (Lys670Asn, Met671Leu) double mutation under the control of the hamster prion protein gene promoter (Hsiao et al. (1996) Science 274:99-102; U.S. Patent No. 5,877,399). Mouse brain samples were prepared at sacrifice by brain removal and knife bisection along the superior sagital sulcus from the cortical surface to the extreme ventral surface. One brain hemi-section from each animal was snap frozen in liquid nitrogen. Frozen brain hemi-sections were weighed and transferred to thick-walled polyallomer centrifuge tubes. A 10x volume (wt:vol) of 70% formic acid was added to each sample. The samples were briefly homogenized over ice, then centrifuged @ 100,000 x g for 1 hr at 4° C. The clear supernatant between the lipid layer and pellet was collected and its volume determined. An 11x volume (vol:vol) 1M Tris Base was added to neutralize the sample to pH range ~ 8 – 8.5, and aliquots were frozen at –80° C until analyzed.

For A387 ELISA analysis of brain samples, A387 antibody was coated onto plates, and the ELISA assay performed essentially as described in Section (1)(b) of this Example. ELISA analysis was also performed using the Human Beta-Amyloid (Abeta) [1-42] Fluorometric ELISA Kit (Biosource, catalog # 88-344), following manufacturers'

directions. The coat antibody in the Biosource Kit is a monoclonal antibody directed against the N-terminus of human Aβ. The detection antibody is a rabbit polyclonal antibody that recognizes human Aβ42, but not human Aβ40 or mouse Aβ. The rabbit antibody is detected using an anti-rabbit IgG-alkaline phosphatase conjugate and a fluorescent substrate. For both the Biosource and the A387 ELISA assays, Aβ42 standard curves were prepared from serial dilutions of Aß42 peptide (Bachem; stored in hexafluoroisopropanol) in C57 brain homogenate.

Results. Using the Biosource Kit ELISA assay, the amount of formic acid-extractable Aβ42 detected in brains of Tg2576 animals was not significantly different from background (C57 brains). However, using A387 antibody and the ELISA protocol described herein, the amount of formic acid-extractable Aβ42 detected in brains of Tg2576 animals was about three-fold higher than background in the linear range of the Aβ42 standard curve.

EXAMPLE 10

Method for administering Aβ monoclonal antibodies to animals and for assessing the effects of the antibodies on Aβ levels and amyloid plaques

Animals. TASD41 transgenic mice, which express human APP751 cDNA containing the London (V717I) and Swedish (K670M/N671L) mutations under the control of the murine Thy-1 gene, are used. The generation and properties of these animals (line 41) are described in Rockenstein *et al.* (2001) *J. Neurosci. Res.* 66:573-582. Briefly, Rockenstein *et al.* showed that TASD41 mice exhibit mature plaques in the frontal cortex as early as 3-4 months of age, and by 5-7 months also exhibit plaques in the hippocampus, thalamus and olfactory region. By ultrastructural and double immunostaining analysis, these plaques were shown to contain dystrophic neuritis immunoreactive with antibodies against APP, snynaptophysin, neurofilament and tau.

As such, the TASD41 mouse is a useful animal model of Alzheimer's disease.

Administration of antibodies. TASD41 mice of either about 4 months of age, or about 8 months of age, are divided into groups of 6-8 age-matched animals. Once a week for 3-6 months, each animal receives an intraperitoneal injection of either 500 μg of A387 antibody in saline, 500 μg of B436 antibody in saline, or 500 μg of control IgG in saline, according to the group.

Aβ ELISA assays. At sacrifice, plasma and brain samples are prepared from each animal as described in Example 9, and ELISA assays performed according to the procedure described in Example 9. A difference in Aβ40 or Aβ42 levels, or of particular Aβ40 or Aβ42 forms, can be detected.

Histopathology. One hemi-brain from each animal is fixed by immersion in 4% paraformaldehyde in PBS (pH 7.4). A series of consecutive 40μM sagittal sections are cut using a Leica Vibratome and stored in cryoprotectant solution at –20°C. Sections are stained with Thioflavine S (which binds amyloid plaques) and with Cresyl Violet, and analyzed under fluorescent and bright field microscopy, respectively. A difference in abundance of amyloid plaques between antibody-treated and Ig-treated animals can be observed.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

What is claimed:

1. A polypeptide, comprising a sequence of amino acids that is selectively reactive with Aβ 42 and preferentially binds to low molecular weight forms of Aβ42.

2. The polypeptide of claim 1, comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387.

3. The polypeptide of claim 1, comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody A387.

4. The polypeptide of claim 1, wherein at least one CDR is selected from the group consisting of amino acids 24-34 of SEQ ID NO:12, amino acids 50-56 of SEQ ID NO:12, amino acids 89-97 of SEQ ID NO:12, amino acids 26-35 of SEQ ID NO:14, amino acids 31-35 of SEQ ID NO:14, amino acids 26-31 of SEQ ID NO:14, amino acids 50-65 of SEQ ID NO:14, amino acids 50-58 of SEQ ID NO:14, and amino acids 98-107 of SEQ ID NO:14.

5. The polypeptide of claim 1, comprising at least a portion of a variable domain of the light chain or the heavy chain of an Aβ antibody.

6. The polypeptide 5, wherein the variable domain is selected from the group consisting of the light chain variable domain of A387, the heavy chain variable domain of A387, a polypeptide with at least 85% identity to the light chain variable domain of A387; a polypeptide with at least 85% identity to the heavy chain variable domain of A387.

7. The polypeptide of claim 1, further comprising a scaffold.

8. The polypeptide of claim 7, wherein the scaffold is a polypeptide scaffold.

9. The polypeptide of claim 7, wherein the scaffold is a human polypeptide scaffold.

10. The polypeptide of claim 7, wherein the scaffold is an antibody scaffold.

11. The polypeptide of claim 10, wherein the antibody scaffold is selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment.

12. The polypeptide of claim 1, further comprising a detectable moiety.

13. The polypeptide of claim 1, further comprising a clearance domain.

14. The polypeptide of claim 13, wherein the clearance domain is a ligand for an Fc receptor.

15. A polypeptide, comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387.

16. The polypeptide of claim 15 comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody A387.

17. The polypeptide of claim 15, wherein at least one CDR is selected from the group consisting of amino acids 24-34 of SEQ ID NO:12, amino acids 50-56 of SEQ ID NO:12, amino acids 89-97 of SEQ ID NO:12, amino acids 26-35 of SEQ ID NO:14, amino acids 31-35 of SEQ ID NO:14, amino acids 26-31 of SEQ ID NO:14, amino acids 50-65 of SEQ ID NO:14, amino acids 50-58 of SEQ ID NO:14, and amino acids 98-107 of SEQ ID NO:14.

18. The polypeptide of any of claims 15-17 further comprising a scaffold.

19. The polypeptide of claim 18 wherein the scaffold comprises a solid support.

20. The polypeptide of claim 18 wherein the scaffold is a polypeptide scaffold.

21. The polypeptide of claim 18, wherein the scaffold is a human polypeptide scaffold.

22. The polypeptide of claim 18, wherein the scaffold is an antibody scaffold.

23. The polypeptide of claim 22, wherein the antibody scaffold is selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment.

24. The polypeptide of any of claims 15-17, wherein the polypeptide is a chimeric polypeptide.

25. The polypeptide of any of claims 15-17, wherein the polypeptide is an antibody.

26.. The polypeptide of any of claims 15-17, further comprising a clearance domain.

27. The polypeptide of claim 26, wherein the clearance domain is a ligand for an Fc receptor.

28. The polypeptide of any of claims 15-17, further comprising a detectable moiety.

29. The polypeptide of claim 16, which comprises amino acids 1-95 of SEQ ID NO:12, or a fragment thereof and/or comprises amino acids 1-97 of SEQ ID NO:14, or a fragment thereof.

30. The polypeptide of claim 29, further comprising one or more joining regions.

31. The polypeptide of claim 30, wherein at least one joining region comprises amino acids 96-107 of SEQ ID NO:12 or amino acids 98-118 of SEQ ID NO:14.

32. The polypeptide of claim 30, wherein at least one joining region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 48, 50, 52, 54, 55, 57, 59, 61, 67, 73, 75, 77, 79, 89 and 91.

33. The polypeptide of claim 29, further comprising one or more constant regions.

34. The polypeptide of claim 33, wherein the constant region is a mouse constant region.

35. The polypeptide of claim 34, wherein the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:63, 65, 69 and 71.

36. The polypeptide of claim 33, wherein the constant region is a human

constant region.

37. The polypeptide of claim 36, wherein the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 83, 85 and 87.

38. The polypeptide of claim 16 comprising the amino acid sequence of SEQ ID NO:97 and/or SEQ ID NO:98.

39. The polypeptide of any of claims 15-17, which is specifically reactive with at least one A□

40. The polypeptide of claim 39, wherein A□is A□□□

41. The polypeptide of claim 39, which binds A□□□ without substantially binding other A□.

42. A polypeptide comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436.

43. The polypeptide of claim 42, comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody B436.

44. The polypeptide of claim 43, wherein at least one CDR is selected from the group consisting of amino acids 24-39 of SEQ ID NO:16, amino acids 55-61 of SEQ ID NO:16, amino acids 94-102 of SEQ ID NO:16, amino acids 26-35 of SEQ ID NO:18, amino acids 31-35 of SEQ ID NO:18, amino acids 26-31 of SEQ ID NO:18, amino acids 50-66 of SEQ ID NO:18, amino acids 50-59 of SEQ ID NO:18, and amino acids 99-103 of SEQ ID NO:18.

45. The polypeptide of any of claims 42-44 further comprising a scaffold.

46. The polypeptide of any of claims 45, wherein the scaffold comprises a solid support.

47. The polypeptide of any of claims 45, wherein the scaffold is a polypeptide scaffold.

48. The polypeptide of claim 45, wherein the scaffold is a human polypeptide scaffold.

49. The polypeptide of claim 45, wherein the scaffold is an antibody scaffold.

50. The polypeptide of claim 49, wherein the antibody scaffold is selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment.

51. The polypeptide of any of claims 42-44, wherein the polypeptide is a chimeric polypeptide.

52. The polypeptide of any of claims 42-44, wherein the polypeptide is an antibody.

53. The polypeptide of any of claims 42-44, further comprising a clearance domain.

54. The polypeptide of claim 53, wherein the clearance domain is a ligand for an Fc receptor.

55. The polypeptide of any of claims 42-44, further comprising a detectable moiety.

56. The polypeptide of claim 43, which comprises amino acids 1-100 of SEQ ID NO:16, or a fragment thereof and/or comprises amino acids 1-98 of SEQ ID NO:18, or a fragment thereof.

57. The polypeptide of claim 56, further comprising one or more joining regions.

58. The polypeptide of claim 57 wherein at least one joining region comprises amino acids 101-112 of SEQ ID NO:16 or amino acids 99-114 of SEQ ID NO:18.

59. The polypeptide of claim 57, wherein at least one joining region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 48, 50, 52, 54, 55, 57, 59, 61, 67, 73, 75, 77, 79, 89 and 91.

60. The polypeptide of claim 56, further comprising one or more constant regions.

61. The polypeptide of claim 60, wherein the constant region is a mouse constant region.

62. The polypeptide of claim 61, wherein the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:63, 65, 69 and 71.

63. The polypeptide of claim 60, wherein the constant region is a human constant region.

64. The polypeptide of claim 63, wherein the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 83, 85 and 87.

65. The polypeptide of claim 43, comprising the amino acid sequence of SEQ ID NO:99 and/or SEQ ID NO:100.

66. The polypeptide of any of claims 42-44 which is specifically reactive with at least one A*β* peptide.

67. A nucleic acid molecule encoding the polypeptide of any of claims 1-41.

68. A nucleic acid molecule encoding the polypeptide of any of claims 42-66.

69. A kit, comprising the polypeptide of any of claims 1-41.

70. A kit, comprising the polypeptide of any of claims 42-66.

71. A method for assessing the presence or amount of A□ in a sample, comprising:

contacting the polypeptide of any of claims 1-14, 39-41 or 66 with the sample under conditions whereby a complex is formed between the polypeptide and A□, and

assessing the presence or amount of the complex in the sample, and thereby determining the presence or amount of A□ in the sample.

72. The method of claim 71, wherein the sample is selected from the group consisting of a cell extract, extracellular medium, plasma, cerebrospinal fluid and brain.

73. The method of claim 71, wherein the presence or amount of the complex

is assessed using an enzyme-linked immunosorbent assay (ELISA).

74. A method, comprising administering to a subject the polypeptide of any of claims 1-66.

75. A method of binding Aβ comprising administering to a subject the polypeptide of any of claims 1-14, 39-41 or 66 to bind A□.

76. The method of claim 74 or 75, wherein the subject has, or is at risk of developing, a disease associated with accumulation of A□.

77. The method of claim 76, wherein the disease is Alzheimer's disease.

78. A method of reducing A□ level in an subject, comprising administering to the subject an effective amount of the polypeptide of any of claims 1-14, 39-41 or 66 to reduce the level of at least one A□peptide.

79. The method of claim 78, wherein the subject has, or is at risk of developing, a disease associated with accumulation of A□.

80. The method of claim 79, wherein the disease is Alzheimer's disease.

81. The method of claim 78, wherein the level of at least one A□peptide in blood or plasma is reduced.

82. The method of claim 78, wherein the level of at least one A□peptide in brain is reduced.

83. A method for assessing presenilin activity, comprising:

contacting a sample containing a presenilin and/or fragment(s) thereof

with a lipoprotein receptor-related protein (LRP) and/or fragment(s) thereof; and

assessing the processing and/or cleavage of the LRP or fragment(s) thereof.

84. A method for identifying an agent that modulates presenilin activity, comprising:

contacting a sample containing a presenilin, and/or fragment(s) thereof, and a lipoprotein receptor-related protein (LRP), and/or fragment(s) thereof with a test agent; and

identifying an agent that alters the processing and/or cleavage of LRP and/or fragment(s) thereof.

85. A method for identifying a candidate agent for treatment or prophylaxis of a disease associated with an altered presenilin, comprising:

contacting a sample that contains an altered presenilin and/or fragment(s) thereof and a lipoprotein receptor-related protein (LRP) and/or fragment(s) thereof with a test agent, wherein the altered presenilin and/or fragment(s) thereof is associated with an altered cleavage and/or processing of LRP and/or fragment(s) thereof; and

identifying a candidate agent that restores LRP cleavage and/or processing to substantially that which occurs in the presence of a presenilin and/or fragment(s) thereof that is not associated with an altered cleavage and/or processing of LRP and/or fragment(s) thereof.

86. A method for modulating LRP, comprising altering the structure, function and/or activity of a presenilin, and/or fragment(s) thereof, in a sample comprising LRP, and/or fragment(s) thereof, and a presenilin, and/or fragment(s) thereof, whereby the LRP is modulated.

87. A method for modulating LRP, comprising contacting a sample comprising an LRP, and/or fragment(s) thereof, and presenilin, and/or fragment(s)

thereof, with an agent that modulates the presenilin and/or fragment(s) thereof or a presenilin-dependent activity, whereby LRP is modulated.

88. A method for identifying an agent that modulates Aβ levels, comprising:

comparing the levels of bound Aβ binding protein in a test sample contacted with the test agent and a control sample not contacted with the test agent; and

identifying an agent as an agent that modulates Aβ levels if the levels of bound Aβ binding protein differ in the test and control samples; wherein

the sample comprises APP or portion(s) thereof; and

the Aβ binding protein comprises the polypeptide of any of claims 1-14, 39-41 or 66.

89. A method for identifying an agent that modulates Aβ42 levels, comprising:

comparing the levels of bound Aβ binding protein in a test sample contacted with the test agent and a control sample not contacted with the test agent; and

identifying an agent as an agent that modulates Aβ42 levels if the levels of bound Aβ binding protein differ in the test and control samples; wherein

the sample comprises APP or portion(s) thereof; and

the Aβ binding protein comprises a sequence of amino acids selected from the group consisting of amino acids 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96 and 1-97 of SEQ ID NO: 12 and 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96 and 1-97 of SEQ ID NO: 14 and any amino acid sequences containing modifications of these amino acid sequences that retain the Aβ binding properties of an antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14.

90. A method for identifying an agent that modulates Aβ levels, comprising:

assessing a test agent that modulates Aβ42 levels to determine if it modulates the level of one or more other Aβ peptides; and

identifying an agent that modulates Aβ42 levels to a greater extent than it modulates the level of one or more other Aβ peptides.

91. A method for identifying an agent that modulates Aβ levels, comprising:

assessing a test agent that modulates Aβ42 levels to determine if it modulates the level of one or more other Aβ peptides; and

identifying an agent that modulates Aβ42 levels and Aβ39 levels.

92. A method for identifying an agent that modulates Aβ levels, comprising:

assessing a test agent that alters the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides to determine if it effects one or more presenilin-dependent activities other than the presenilin-dependent processing of APP or portion(s) thereof; and

identifying an agent that modulates Aβ levels without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP.

93. A method for identifying an agent that modulates Aβ levels, comprising:

assessing a test agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides to determine if it effects the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof other than APP; and

identifying an agent that modulates Aβ levels without substantially altering the cleavage and/or processing of the presenilin substrate and/or portion(s) thereof that is other than APP.

94. A method for identifying an agent that modulates Aβ levels, comprising:

assessing a test agent that modulates the cleavage of APP that produces one or more Aβ peptides, the processing of APP, the processing of Aβ and/or the level of one or more Aβ peptides to determine if it effects the cleavage and/or processing of LRP

and/or portion(s) thereof; and

identifying an agent that modulates Aβ levels without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof.

95. A system for use in assessing presenilin activity, comprising:

a source of presenilin activity;

a source of LRP (and/or portion(s) thereof); and

a reagent for determining LRP protein composition.

96. A kit comprising:

a reagent for assessing cleavage of APP that produces one or more Aβ peptides, APP processing, Aβ processing and/or Aβ levels; and

a reagent for assessing cleavage and/or processing of a presenilin substrate.

97 A method for identifying a candidate agent for the treatment or prophylaxis of a disease, comprising:

contacting a sample that contains an altered test protein, and/or portion(s) thereof, and APP, and/or portion(s) thereof, with a test agent, wherein the altered protein is associated with altered Aβ42 production, catabolism, processing and/or Aβ42 levels; and

identifying a candidate agent that restores Aβ production, catabolism, processing and/or Aβ levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered Aβ42 production, catabolism, processing and/or Aβ42 levels without substantially altering the level of one or more other Aβ peptides.

98. A method for identifying a candidate agent for the treatment or prophylaxis of a disease, comprising:

contacting a sample that contains an altered test protein, and/or portion(s)

thereof, and APP, and/or portion(s) thereof, with a test agent, wherein the altered protein is associated with altered $A\beta$ production, catabolism, processing and/or $A\beta$ levels; and

identifying a candidate agent that restores $A\beta$ production, catabolism, processing and/or $A\beta$ levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered $A\beta$ production, catabolism, processing and/or $A\beta$ levels without substantially altering (a) one or more presenilin-dependent activities other than the presenilin-dependent processing of APP, (b) the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP and/or (c) the cleavage and/or processing of LRP and/or portion(s) thereof.

99. A method for identifying an agent that modulates $A\beta$ levels, comprising:

assessing a test agent that alters the cleavage of APP that produces one or more $A\beta$ peptides, the processing of APP, the processing of $A\beta$ and/or the level of one or more $A\beta$ peptides to determine if it affects one or more presenilin-dependent activities other than the presenilin-dependent processing of APP or portion(s) thereof that produces one or more $A\beta$ peptides; and

identifying an agent that modulates $A\beta$ levels without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP or portion(s) thereof that produces one or more $A\beta$ peptides.

100. A method for identifying an agent that modulates $A\beta$ levels, comprising:

assessing a test agent that modulates the cleavage of APP that produces one or more $A\beta$ peptides, the processing of APP, the processing of $A\beta$ and/or the level of one or more $A\beta$ peptides to determine if it affects the cleavage and/or processing of APP and/or portion(s) thereof other than the processing of APP or portion(s) thereof that produces one or more $A\beta$ peptides; and

identifying an agent that modulates $A\beta$ levels without substantially altering the cleavage and/or processing of APP and/or portion(s) thereof other than the processing of APP or portion(s) thereof that produces one or more $A\beta$ peptides.

SEQUENCE LISTING

<110> Kounnas, Maria
Patrick, Aaron
Wagner, Steven
Velicelebi, Gonul

<120> METHODS AND COMPOSITIONS FOR MODULATING AMYLOID BETA

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3560
aatacattct tggaggagc
3579

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<211> 770
<212> PRT
<213> Homo sapiens

<400> 2
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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20   25   30
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35   40   45
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50   55   60
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65   70   75   80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85   90   95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100   105   110
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115   120   125
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130   135   140
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145   150   155   160
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165   170   175
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180   185   190
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195   200   205
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210   215   220
Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225   230   235   240
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245   250   255
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260   265   270
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg

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            275                 280                 285
Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
        290                 295                 300
Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305                 310                 315                 320
Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu Tyr
                325                 330                 335
Cys Met Ala Val Cys Gly Ser Ala Met Ser Gln Ser Leu Leu Lys Thr
            340                 345                 350
Thr Gln Glu Pro Leu Ala Arg Asp Pro Val Lys Leu Pro Thr Thr Ala
        355                 360                 365
Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp
    370                 375                 380
Glu Asn Glu His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala
385                 390                 395                 400
Lys His Arg Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala
                405                 410                 415
Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile
            420                 425                 430
Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn
        435                 440                 445
Glu Arg Gln Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met
    450                 455                 460
Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu
465                 470                 475                 480
Gln Ala Val Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys
                485                 490                 495
Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe
            500                 505                 510
Glu His Val Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser
        515                 520                 525
Gln Val Met Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser
    530                 535                 540
Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp
545                 550                 555                 560
Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val
                565                 570                 575
Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala
            580                 585                 590
Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro
        595                 600                 605
Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe
    610                 615                 620
Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val
625                 630                 635                 640
Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser
                645                 650                 655
Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp
            660                 665                 670
Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu
        675                 680                 685
Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly
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    690                 695                 700
Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu
705                 710                 715                 720
Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val
                725                 730                 735
Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met
            740                 745                 750
Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met
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Gln Asn
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<210> 3
<211> 248
<212> DNA
<213> Homo sapiens

<220>
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<222> (51)...(179)
<223> nucleotide sequence encoding Human amyloid-beta
      precursor protein

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56
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                                                        1

gaa ttc cga cat gac tca gga tat gaa gtt cat cat caa aaa ttg gtg
104
Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val
         5                  10                  15

ttc ttt gca gaa gat gtg ggt tca aac aaa ggt gca atc att gga ctc
152
Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu
     20                  25                  30

atg gtg ggc ggt gtt gtc ata gcg aca gtgatcgtca tcaccttggt
199
Met Val Gly Gly Val Val Ile Ala Thr
 35                  40

gatgctgaag aagaaacagt acacatccat tcatcatggt gtggtggag
248

<210> 4
<211> 43
<212> PRT
<213> Homo sapiens
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<400> 4
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15
Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30
Gly Leu Met Val Gly Gly Val Val Ile Ala Thr
35 40

<210> 5
<211> 2765
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (249)...(1649)
<223> nucleotide sequence encoding human Presnilin-1
(hPS1)

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60
gaaggaacct gagctacgag ccgcggcggc agcggggcgg cggggaagcg tatacctaat
120
ctgggagcct gcaagtgaca acagcctttg cggtccttag acagcttggc ctggaggaga
180
acacatgaaa gaaagaacct caagaggctt tgttttctgt gaaacagtat ttctatacag
240
ttgctcca atg aca gag tta cct gca ccg ttg tcc tac ttc cag aat gca
290
Met Thr Glu Leu Pro Ala Pro Leu Ser Tyr Phe Gln Asn Ala
1 5 10

cag atg tct gag gac aac cac ctg agc aat act gta cgt agc cag aat
338
Gln Met Ser Glu Asp Asn His Leu Ser Asn Thr Val Arg Ser Gln Asn
15 20 25 30

gac aat aga gaa cgg cag gag cac aac gac aga cgg agc ctt ggc cac
386
Asp Asn Arg Glu Arg Gln Glu His Asn Asp Arg Arg Ser Leu Gly His
35 40 45

cct gag cca tta tct aat gga cga ccc cag ggt aac tcc cgg cag gtg
434
Pro Glu Pro Leu Ser Asn Gly Arg Pro Gln Gly Asn Ser Arg Gln Val
50 55 60

gtg gag caa gat gag gaa gaa gat gag gag ctg aca ttg aaa tat ggc
482
Val Glu Gln Asp Glu Glu Glu Asp Glu Glu Leu Thr Leu Lys Tyr Gly
65 70 75

gcc aag cat gtg atc atg ctc ttt gtc cct gtg act ctc tgc atg gtg 530
Ala Lys His Val Ile Met Leu Phe Val Pro Val Thr Leu Cys Met Val
80 85 90

gtg gtc gtg gct acc att aag tca gtc agc ttt tat acc cgg aag gat 578
Val Val Val Ala Thr Ile Lys Ser Val Ser Phe Tyr Thr Arg Lys Asp
95 100 105 110

ggg cag cta atc tat acc cca ttc aca gaa gat acc gag act gtg ggc 626
Gly Gln Leu Ile Tyr Thr Pro Phe Thr Glu Asp Thr Glu Thr Val Gly
115 120 125

cag aga gcc ctg cac tca att ctg aat gct gcc atc atg atc agt gtc 674
Gln Arg Ala Leu His Ser Ile Leu Asn Ala Ala Ile Met Ile Ser Val
130 135 140

att gtt gtc atg act atc ctc ctg gtg gtt ctg tat aaa tac agg tgc 722
Ile Val Val Met Thr Ile Leu Leu Val Val Leu Tyr Lys Tyr Arg Cys
145 150 155

tat aag gtc atc cat gcc tgg ctt att ata tca tct cta ttg ttg ctg 770
Tyr Lys Val Ile His Ala Trp Leu Ile Ile Ser Ser Leu Leu Leu Leu
160 165 170

ttc ttt ttt tca ttc att tac ttg ggg gaa gtg ttt aaa acc tat aac 818
Phe Phe Phe Ser Phe Ile Tyr Leu Gly Glu Val Phe Lys Thr Tyr Asn
175 180 185 190

gtt gct gtg gac tac att act gtt gca ctc ctg atc tgg aat ttt ggt 866
Val Ala Val Asp Tyr Ile Thr Val Ala Leu Leu Ile Trp Asn Phe Gly
195 200 205

gtg gtg gga atg att tcc att cac tgg aaa ggt cca ctt cga ctc cag 914
Val Val Gly Met Ile Ser Ile His Trp Lys Gly Pro Leu Arg Leu Gln
210 215 220

cag gca tat ctc att atg att agt gcc ctc atg gcc ctg gtg ttt atc 962
Gln Ala Tyr Leu Ile Met Ile Ser Ala Leu Met Ala Leu Val Phe Ile
225 230 235

aag tac ctc cct gaa tgg act gcg tgg ctc atc ttg gct gtg att tca
1010
Lys Tyr Leu Pro Glu Trp Thr Ala Trp Leu Ile Leu Ala Val Ile Ser
240 245 250

gta tat gat tta gtg gct gtt ttg tgt ccg aaa ggt cca ctt cgt atg
1058
Val Tyr Asp Leu Val Ala Val Leu Cys Pro Lys Gly Pro Leu Arg Met
255 260 265 270

ctg gtt gaa aca gct cag gag aga aat gaa acg ctt ttt cca gct ctc
1106
Leu Val Glu Thr Ala Gln Glu Arg Asn Glu Thr Leu Phe Pro Ala Leu
275 280 285

att tac tcc tca aca atg gtg tgg ttg gtg aat atg gca gaa gga gac
1154
Ile Tyr Ser Ser Thr Met Val Trp Leu Val Asn Met Ala Glu Gly Asp
290 295 300

ccg gaa gct caa agg aga gta tcc aaa aat tcc aag tat aat gca gaa
1202
Pro Glu Ala Gln Arg Arg Val Ser Lys Asn Ser Lys Tyr Asn Ala Glu
305 310 315

agc aca gaa agg gag tca caa gac act gtt gca gag aat gat gat ggc
1250
Ser Thr Glu Arg Glu Ser Gln Asp Thr Val Ala Glu Asn Asp Asp Gly
320 325 330

ggg ttc agt gag gaa tgg gaa gcc cag agg gac agt cat cta ggg cct
1298
Gly Phe Ser Glu Glu Trp Glu Ala Gln Arg Asp Ser His Leu Gly Pro
335 340 345 350

cat cgc tct aca cct gag tca cga gct gct gtc cag gaa ctt tcc agc
1346
His Arg Ser Thr Pro Glu Ser Arg Ala Ala Val Gln Glu Leu Ser Ser
355 360 365

agt atc ctc gct ggt gaa gac cca gag gaa agg gga gta aaa ctt gga
1394
Ser Ile Leu Ala Gly Glu Asp Pro Glu Glu Arg Gly Val Lys Leu Gly
370 375 380

ttg gga gat ttc att ttc tac agt gtt ctg gtt ggt aaa gcc tca gca
1442
Leu Gly Asp Phe Ile Phe Tyr Ser Val Leu Val Gly Lys Ala Ser Ala
385 390 395

aca gcc agt gga gac tgg aac aca acc ata gcc tgt ttc gta gcc ata
1490

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Thr Ala Ser Gly Asp Trp Asn Thr Thr Ile Ala Cys Phe Val Ala Ile
    400                 405                 410

tta att ggt ttg tgc ctt aca tta tta ctc ctt gcc att ttc aag aaa
1538
Leu Ile Gly Leu Cys Leu Thr Leu Leu Leu Leu Ala Ile Phe Lys Lys
415                 420                 425                 430

gca ttg cca gct ctt cca atc tcc atc acc ttt ggg ctt gtt ttc tac
1586
Ala Leu Pro Ala Leu Pro Ile Ser Ile Thr Phe Gly Leu Val Phe Tyr
                435                 440                 445

ttt gcc aca gat tat ctt gta cag cct ttt atg gac caa tta gca ttc
1634
Phe Ala Thr Asp Tyr Leu Val Gln Pro Phe Met Asp Gln Leu Ala Phe
            450                 455                 460

cat caa ttt tat atc tagcatattt gcggttagaa tcccatggat gtttcttctt
1689
His Gln Phe Tyr Ile
        465

tgactataac caaatctggg gaggacaaag gtgattttcc tgtgtccaca tctaacaaag
1749
tcaagattcc cggctggact tttgcagctt ccttccaagt cttcctgacc accttgcact
1809
attggacttt ggaaggaggt gcctatagaa aacgattttg aacatacttc atcgcagtgg
1869
actgtgtccc tcggtgcaga aactaccaga tttgagggac gaggtcaagg agatatgata
1929
ggcccggaag ttgctgtgcc ccatcagcag cttgacgcgt ggtcacagga cgatttcact
1989
gacactgcga actctcagga ctaccggtta ccaagaggtt aggtgaagtg gtttaaacca
2049
aacggaactc ttcatcttaa actacacgtt gaaaatcaac ccaataattc tgtattaact
2109
gaattctgaa cttttcagga ggtactgtga ggaagagcag gcaccagcag cagaatgggg
2169
aatggagagg tgggcagggg ttccagcttc cctttgattt tttgctgcag actcatcctt
2229
tttaaatgag acttgttttc ccctctcttt gagtcaagtc aaatatgtag attgcctttg
2289
gcaattcttc ttctcaagca ctgacactca ttaccgtctg tgattgccat ttcttcccaa
2349
ggccagtctg aacctgaggt tgctttatcc taaaagtttt aacctcaggt tccaaattca
2409
gtaaattttg gaaacagtac agctatttct catcaattct ctatcatgtt gaagtcaaat
2469
ttggattttc caccaaattc tgaatttgta gacatacttg tacgctcact tgcccccaga
2529
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tgcctcctct gtcctcattc ttctctccca cacaagcagt ctttttctac agccagtaag 2589
gcagctctgt crtggtagca gatggtccca ttattctagg gtcttactct ttgtatgatg 2649
aaaagaatgt gttatgaatc ggtgctgtca gccctgctgt cagaccttct tccacagcaa 2709
atgagatgta tgcccaaagc ggtagaatta aagaagagta aaatggctgt tgaagc 2765

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<211> 467
<212> PRT
<213> Homo sapiens

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20 25 30
Arg Glu Arg Gln Glu His Asn Asp Arg Arg Ser Leu Gly His Pro Glu
35 40 45
Pro Leu Ser Asn Gly Arg Pro Gln Gly Asn Ser Arg Gln Val Val Glu
50 55 60
Gln Asp Glu Glu Glu Asp Glu Glu Leu Thr Leu Lys Tyr Gly Ala Lys
65 70 75 80
His Val Ile Met Leu Phe Val Pro Val Thr Leu Cys Met Val Val Val
85 90 95
Val Ala Thr Ile Lys Ser Val Ser Phe Tyr Thr Arg Lys Asp Gly Gln
100 105 110
Leu Ile Tyr Thr Pro Phe Thr Glu Asp Thr Glu Thr Val Gly Gln Arg
115 120 125
Ala Leu His Ser Ile Leu Asn Ala Ala Ile Met Ile Ser Val Ile Val
130 135 140
Val Met Thr Ile Leu Leu Val Val Leu Tyr Lys Tyr Arg Cys Tyr Lys
145 150 155 160
Val Ile His Ala Trp Leu Ile Ile Ser Ser Leu Leu Leu Leu Phe Phe
165 170 175
Phe Ser Phe Ile Tyr Leu Gly Glu Val Phe Lys Thr Tyr Asn Val Ala
180 185 190
Val Asp Tyr Ile Thr Val Ala Leu Leu Ile Trp Asn Phe Gly Val Val
195 200 205
Gly Met Ile Ser Ile His Trp Lys Gly Pro Leu Arg Leu Gln Gln Ala
210 215 220
Tyr Leu Ile Met Ile Ser Ala Leu Met Ala Leu Val Phe Ile Lys Tyr
225 230 235 240
Leu Pro Glu Trp Thr Ala Trp Leu Ile Leu Ala Val Ile Ser Val Tyr
245 250 255
Asp Leu Val Ala Val Leu Cys Pro Lys Gly Pro Leu Arg Met Leu Val
260 265 270
Glu Thr Ala Gln Glu Arg Asn Glu Thr Leu Phe Pro Ala Leu Ile Tyr
275 280 285
Ser Ser Thr Met Val Trp Leu Val Asn Met Ala Glu Gly Asp Pro Glu

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    290                 295                 300
Ala Gln Arg Arg Val Ser Lys Asn Ser Lys Tyr Asn Ala Glu Ser Thr
305                 310                 315                 320
Glu Arg Glu Ser Gln Asp Thr Val Ala Glu Asn Asp Asp Gly Gly Phe
                325                 330                 335
Ser Glu Glu Trp Glu Ala Gln Arg Asp Ser His Leu Gly Pro His Arg
            340                 345                 350
Ser Thr Pro Glu Ser Arg Ala Ala Val Gln Glu Leu Ser Ser Ser Ile
        355                 360                 365
Leu Ala Gly Glu Asp Pro Glu Glu Arg Gly Val Lys Leu Gly Leu Gly
    370                 375                 380
Asp Phe Ile Phe Tyr Ser Val Leu Val Gly Lys Ala Ser Ala Thr Ala
385                 390                 395                 400
Ser Gly Asp Trp Asn Thr Thr Ile Ala Cys Phe Val Ala Ile Leu Ile
                405                 410                 415
Gly Leu Cys Leu Thr Leu Leu Leu Leu Ala Ile Phe Lys Lys Ala Leu
            420                 425                 430
Pro Ala Leu Pro Ile Ser Ile Thr Phe Gly Leu Val Phe Tyr Phe Ala
        435                 440                 445
Thr Asp Tyr Leu Val Gln Pro Phe Met Asp Gln Leu Ala Phe His Gln
    450                 455                 460
Phe Tyr Ile
465

<210> 7
<211> 2236
<212> DNA
<213> Homo sapiens

<220>
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<222> (368)...(1714)
<223> nucleotide sequence encoding human presnilin-2
      (PSEN2)

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120
gaggccacag agctaaagtg acttttccca aggtcgccca gcgaggacgt gggacttctc
180
agacgtcagg agagtgatgt gagggagctg tgtgaccata gaaagtgacg tgttaaaaac
240
cagcgctgcc ctctttgaaa gccagggagc atcattcatt tagcctgctg agaagaagaa
300
accaagtgtc cgggattcag acctctctgc ggccccaagt gttcgtggtg cttccagagg
360
cagggct atg ctc aca ttc atg gcc tct gac agc gag gaa gaa gtg tgt
409
        Met Leu Thr Phe Met Ala Ser Asp Ser Glu Glu Glu Val Cys
         1               5                  10
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gat gag cgg acg tcc cta atg tcg gcc gag agc ccc acg ccg cgc tcc
457
Asp Glu Arg Thr Ser Leu Met Ser Ala Glu Ser Pro Thr Pro Arg Ser
15 20 25 30

tgc cag gag ggc agg cag ggc cca gag gat gga gag aac act gcc cag
505
Cys Gln Glu Gly Arg Gln Gly Pro Glu Asp Gly Glu Asn Thr Ala Gln
35 40 45

tgg aga agc cag gag aac gag gag gac ggt gag gag gac cct gac cgc
553
Trp Arg Ser Gln Glu Asn Glu Glu Asp Gly Glu Glu Asp Pro Asp Arg
50 55 60

tat gtc tgt agt ggg gtt ccc ggg cgg ccg cca ggc ctg gag gaa gag
601
Tyr Val Cys Ser Gly Val Pro Gly Arg Pro Pro Gly Leu Glu Glu Glu
65 70 75

ctg acc ctc aaa tac gga gcg aag cac gtg atc atg ctg ttt gtg cct
649
Leu Thr Leu Lys Tyr Gly Ala Lys His Val Ile Met Leu Phe Val Pro
80 85 90

gtc act ctg tgc atg atc gtg gtg gta gcc acc atc aag tct gtg cgc
697
Val Thr Leu Cys Met Ile Val Val Val Ala Thr Ile Lys Ser Val Arg
95 100 105 110

ttc tac aca gag aag aat gga cag ctc atc tac acg aca ttc act gag
745
Phe Tyr Thr Glu Lys Asn Gly Gln Leu Ile Tyr Thr Thr Phe Thr Glu
115 120 125

gac aca ccc tcg gtg ggc cag cgc ctc ctc aac tcc gtg ctg aac acc
793
Asp Thr Pro Ser Val Gly Gln Arg Leu Leu Asn Ser Val Leu Asn Thr
130 135 140

ctc atc atg atc agc gtc atc gtg gtt atg acc atc ttc ttg gtg gtg
841
Leu Ile Met Ile Ser Val Ile Val Val Met Thr Ile Phe Leu Val Val
145 150 155

ctc tac aag tac cgc tgc tac aag ttc atc cat ggc tgg ttg atc atg
889
Leu Tyr Lys Tyr Arg Cys Tyr Lys Phe Ile His Gly Trp Leu Ile Met
160 165 170

tct tca ctg atg ctg ctg ttc ctc ttc acc tat atc tac ctt ggg gaa
937
Ser Ser Leu Met Leu Leu Phe Leu Phe Thr Tyr Ile Tyr Leu Gly Glu
175 180 185 190

gtg ctc aag acc tac aat gtg gcc atg gac tac ccc acc ctc ttg ctg
985
Val Leu Lys Thr Tyr Asn Val Ala Met Asp Tyr Pro Thr Leu Leu Leu
195 200 205

act gtc tgg aac ttc ggg gca gtg ggc atg gtg tgc atc cac tgg aag
1033
Thr Val Trp Asn Phe Gly Ala Val Gly Met Val Cys Ile His Trp Lys
210 215 220

ggc cct ctg gtg ctg cag cag gcc tac ctc atc atg atc agt gcg ctc
1081
Gly Pro Leu Val Leu Gln Gln Ala Tyr Leu Ile Met Ile Ser Ala Leu
225 230 235

atg gcc cta gtg ttc atc aag tac ctc cca gag tgg tcc gcg tgg gtc
1129
Met Ala Leu Val Phe Ile Lys Tyr Leu Pro Glu Trp Ser Ala Trp Val
240 245 250

atc ctg ggc gcc atc tct gtg tat gat ctc gtg gct gtg ctg tgt ccc
1177
Ile Leu Gly Ala Ile Ser Val Tyr Asp Leu Val Ala Val Leu Cys Pro
255 260 265 270

aaa ggg cct ctg aga atg ctg gta gaa act gcc cag gag aga aat gag
1225
Lys Gly Pro Leu Arg Met Leu Val Glu Thr Ala Gln Glu Arg Asn Glu
275 280 285

ccc ata ttc cct gcc ctg ata tac tca tct gcc atg gtg tgg acg gtt
1273
Pro Ile Phe Pro Ala Leu Ile Tyr Ser Ser Ala Met Val Trp Thr Val
290 295 300

ggc atg gcg aag ctg gac ccc tcc tct cag ggt gcc ctc cag ctc ccc
1321
Gly Met Ala Lys Leu Asp Pro Ser Ser Gln Gly Ala Leu Gln Leu Pro
305 310 315

tac gac ccg gag atg gaa gaa gac tcc tat gac agt ttt ggg gag cct
1369
Tyr Asp Pro Glu Met Glu Glu Asp Ser Tyr Asp Ser Phe Gly Glu Pro
320 325 330

tca tac ccc gaa gtc ttt gag cct ccc ttg act ggc tac cca ggg gag
1417

Ser Tyr Pro Glu Val Phe Glu Pro Pro Leu Thr Gly Tyr Pro Gly Glu
335 340 345 350

gag ctg gag gaa gag gag gaa agg ggc gtg aag ctt ggc ctc ggg gac
1465
Glu Leu Glu Glu Glu Glu Glu Arg Gly Val Lys Leu Gly Leu Gly Asp
355 360 365

ttc atc ttc tac agt gtg ctg gtg ggc aag gcg gct gcc acg ggc agc
1513
Phe Ile Phe Tyr Ser Val Leu Val Gly Lys Ala Ala Ala Thr Gly Ser
370 375 380

ggg gac tgg aat acc acg ctg gcc tgc ttc gtg gcc atc ctc att ggc
1561
Gly Asp Trp Asn Thr Thr Leu Ala Cys Phe Val Ala Ile Leu Ile Gly
385 390 395

ttg tgt ctg acc ctc ctg ctg ctt gct gtg ttc aag aag gcg ctg ccc
1609
Leu Cys Leu Thr Leu Leu Leu Leu Ala Val Phe Lys Lys Ala Leu Pro
400 405 410

gcc ctc ccc atc tcc atc acg ttc ggg ctc atc ttt tac ttc tcc acg
1657
Ala Leu Pro Ile Ser Ile Thr Phe Gly Leu Ile Phe Tyr Phe Ser Thr
415 420 425 430

gac aac ctg gtg cgg ccg ttc atg gac acc ctg gcc tcc cat cag ctc
1705
Asp Asn Leu Val Arg Pro Phe Met Asp Thr Leu Ala Ser His Gln Leu
435 440 445

tac atc tga gggacatggt gtgccacagg ctgcaagctg cagggaattt
1754
Tyr Ile

tcattggatg cagttgtata gttttacact ctagtgccat atatttttaa gacttttctt
1814
tccttaaaaa ataaagtacg tgtttacttg gtgaggagga ggcagaacca gctctttggt
1874
gccagctgtt tcatcaccag actttggctc ccgctttggg gagcgcctcg cttcacggac
1934
aggaagcaca gcaggtttat ccagatgaac tgagaaggtc agattagggc ggggagaaga
1994
gcatccggca tgagggctga gatgcgcaaa gagtgtgctc gggagtggcc cctggcacct
2054
gggtgctctg gctggagagg aaaagccagt tccctacgag gagtgttccc aatgctttgt
2114
ccatgatgtc cttgttattt tattgccttt agaaactgag tcctgttctt gttacggcag
2174

tcacactgct gggaagtggc ttaatagtaa tatcaataaa tagatgagtc ctgttagaaa
2234
aa
2236

<210> 8
<211> 448
<212> PRT
<213> Homo sapiens

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35 40 45
Ser Gln Glu Asn Glu Glu Asp Gly Glu Glu Asp Pro Asp Arg Tyr Val
50 55 60
Cys Ser Gly Val Pro Gly Arg Pro Pro Gly Leu Glu Glu Glu Leu Thr
65 70 75 80
Leu Lys Tyr Gly Ala Lys His Val Ile Met Leu Phe Val Pro Val Thr
85 90 95
Leu Cys Met Ile Val Val Val Ala Thr Ile Lys Ser Val Arg Phe Tyr
100 105 110
Thr Glu Lys Asn Gly Gln Leu Ile Tyr Thr Thr Phe Thr Glu Asp Thr
115 120 125
Pro Ser Val Gly Gln Arg Leu Leu Asn Ser Val Leu Asn Thr Leu Ile
130 135 140
Met Ile Ser Val Ile Val Val Met Thr Ile Phe Leu Val Val Leu Tyr
145 150 155 160
Lys Tyr Arg Cys Tyr Lys Phe Ile His Gly Trp Leu Ile Met Ser Ser
165 170 175
Leu Met Leu Leu Phe Leu Phe Thr Tyr Ile Tyr Leu Gly Glu Val Leu
180 185 190
Lys Thr Tyr Asn Val Ala Met Asp Tyr Pro Thr Leu Leu Leu Thr Val
195 200 205
Trp Asn Phe Gly Ala Val Gly Met Val Cys Ile His Trp Lys Gly Pro
210 215 220
Leu Val Leu Gln Gln Ala Tyr Leu Ile Met Ile Ser Ala Leu Met Ala
225 230 235 240
Leu Val Phe Ile Lys Tyr Leu Pro Glu Trp Ser Ala Trp Val Ile Leu
245 250 255
Gly Ala Ile Ser Val Tyr Asp Leu Val Ala Val Leu Cys Pro Lys Gly
260 265 270
Pro Leu Arg Met Leu Val Glu Thr Ala Gln Glu Arg Asn Glu Pro Ile
275 280 285
Phe Pro Ala Leu Ile Tyr Ser Ser Ala Met Val Trp Thr Val Gly Met
290 295 300
Ala Lys Leu Asp Pro Ser Ser Gln Gly Ala Leu Gln Leu Pro Tyr Asp
305 310 315 320
Pro Glu Met Glu Glu Asp Ser Tyr Asp Ser Phe Gly Glu Pro Ser Tyr

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                325                 330                 335
Pro Glu Val Phe Glu Pro Pro Leu Thr Gly Tyr Pro Gly Glu Glu Leu
            340                 345                 350
Glu Glu Glu Glu Glu Arg Gly Val Lys Leu Gly Leu Gly Asp Phe Ile
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Phe Tyr Ser Val Leu Val Gly Lys Ala Ala Ala Thr Gly Ser Gly Asp
    370                 375                 380
Trp Asn Thr Thr Leu Ala Cys Phe Val Ala Ile Leu Ile Gly Leu Cys
385                 390                 395                 400
Leu Thr Leu Leu Leu Leu Ala Val Phe Lys Lys Ala Leu Pro Ala Leu
                405                 410                 415
Pro Ile Ser Ile Thr Phe Gly Leu Ile Phe Tyr Phe Ser Thr Asp Asn
            420                 425                 430
Leu Val Arg Pro Phe Met Asp Thr Leu Ala Ser His Gln Leu Tyr Ile
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<223> nucleotide sequence encoding Human low density
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120
cgcctcctcc caattgtgca tttttgcagc cggaggcggc tccgagatgg ggctgtgagc
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240
tggatttcgg ggcagggggc gcacccccgt cagcaggccc tccccaaggg gctcggaact
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ctacctcttc acccacgccc ctggtgcgct ttgccgaagg aaagaataag aacagagaag
360
gaggaggggg aaaggaggaa aagggggacc ccccaactgg gggggtgaa ggagagaagt
420
agcaggacca gaggggaagg ggctgctgct tgcatcagcc cacacc atg ctg acc
475
                                                   Met Leu Thr
                                                    1

ccg ccg ttg ctc ctg ctg ctg ccc ctg ctc tca gct ctg gtc gcg gcg
523
Pro Pro Leu Leu Leu Leu Leu Pro Leu Leu Ser Ala Leu Val Ala Ala
     5                  10                  15
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gct atc gac gcc cct aag act tgc agc ccc aag cag ttt gcc tgc aga
571
Ala Ile Asp Ala Pro Lys Thr Cys Ser Pro Lys Gln Phe Ala Cys Arg
 20                 25                  30                  35

gat caa ata acc tgt atc tca aag ggc tgg cgg tgc gac ggt gag agg
619
Asp Gln Ile Thr Cys Ile Ser Lys Gly Trp Arg Cys Asp Gly Glu Arg
                40                  45                  50

gac tgc cca gac gga tct gac gag gcc cct gag att tgt cca cag agt
667
Asp Cys Pro Asp Gly Ser Asp Glu Ala Pro Glu Ile Cys Pro Gln Ser
            55                  60                  65

aag gcc cag cga tgc cag cca aac gag cat aac tgc ctg ggt act gag
715
Lys Ala Gln Arg Cys Gln Pro Asn Glu His Asn Cys Leu Gly Thr Glu
        70                  75                  80

ctg tgt gtt ccc atg tcc cgc ctc tgc aat ggg gtc cag gac tgc atg
763
Leu Cys Val Pro Met Ser Arg Leu Cys Asn Gly Val Gln Asp Cys Met
    85                  90                  95

gac ggc tca gat gag ggg ccc cac tgc cga gag ctc caa ggc aac tgc
811
Asp Gly Ser Asp Glu Gly Pro His Cys Arg Glu Leu Gln Gly Asn Cys
100                 105                 110                 115

tct cgc ctg ggc tgc cag cac cat tgt gtc ccc aca ctc gat ggg ccc
859
Ser Arg Leu Gly Cys Gln His His Cys Val Pro Thr Leu Asp Gly Pro
                120                 125                 130

acc tgc tac tgc aac agc agc ttt cag ctt cag gca gat ggc aag acc
907
Thr Cys Tyr Cys Asn Ser Ser Phe Gln Leu Gln Ala Asp Gly Lys Thr
            135                 140                 145

tgc aaa gat ttt gat gag tgc tca gtg tac ggc acc tgc agc cag cta
955
Cys Lys Asp Phe Asp Glu Cys Ser Val Tyr Gly Thr Cys Ser Gln Leu
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tgc acc aac aca gac ggc tcc ttc ata tgt ggc tgt gtt gaa gga tac
1003
Cys Thr Asn Thr Asp Gly Ser Phe Ile Cys Gly Cys Val Glu Gly Tyr
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ctc ctg cag ccg gat aac cgc tcc tgc aag gcc aag aac gag cca gta
1051
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Leu Leu Gln Pro Asp Asn Arg Ser Cys Lys Ala Lys Asn Glu Pro Val
180 185 190 195

gac cgg ccc cct gtg ctg ttg ata gcc aac tcc cag aac atc ttg gcc
1099
Asp Arg Pro Pro Val Leu Leu Ile Ala Asn Ser Gln Asn Ile Leu Ala
200 205 210

acg tac ctg agt ggg gcc cag gtg tct acc atc aca cct acg agc acg
1147
Thr Tyr Leu Ser Gly Ala Gln Val Ser Thr Ile Thr Pro Thr Ser Thr
215 220 225

cgg cag acc aca gcc atg gac ttc agc tat gcc aac gag acc gta tgc
1195
Arg Gln Thr Thr Ala Met Asp Phe Ser Tyr Ala Asn Glu Thr Val Cys
230 235 240

tgg gtg cat gtt ggg gac agt gct gct cag acg cag ctc aag tgt gcc
1243
Trp Val His Val Gly Asp Ser Ala Ala Gln Thr Gln Leu Lys Cys Ala
245 250 255

cgc atg cct ggc cta aag ggc ttc gtg gat gag cac acc atc aac atc
1291
Arg Met Pro Gly Leu Lys Gly Phe Val Asp Glu His Thr Ile Asn Ile
260 265 270 275

tcc ctc agt ctg cac cac gtg gaa cag atg gcc atc gac tgg ctg aca
1339
Ser Leu Ser Leu His His Val Glu Gln Met Ala Ile Asp Trp Leu Thr
280 285 290

ggc aac ttc tac ttt gtg gat gac atc gat gat agg atc ttt gtc tgc
1387
Gly Asn Phe Tyr Phe Val Asp Asp Ile Asp Asp Arg Ile Phe Val Cys
295 300 305

aac aga aat ggg gac aca tgt gtc aca ttg cta gac ctg gaa ctc tac
1435
Asn Arg Asn Gly Asp Thr Cys Val Thr Leu Leu Asp Leu Glu Leu Tyr
310 315 320

aac ccc aag ggc att gcc ctg gac cct gcc atg ggg aag gtg ttt ttc
1483
Asn Pro Lys Gly Ile Ala Leu Asp Pro Ala Met Gly Lys Val Phe Phe
325 330 335

act gac tat ggg cag atc cca aag gtg gaa cgc tgt gac atg gat ggg
1531
Thr Asp Tyr Gly Gln Ile Pro Lys Val Glu Arg Cys Asp Met Asp Gly
340 345 350 355

cag aac cgc acc aag ctc gtc gac agc aag att gtg ttt cct cat ggc
1579
Gln Asn Arg Thr Lys Leu Val Asp Ser Lys Ile Val Phe Pro His Gly
360 365 370

atc acg ctg gac ctg gtc agc cgc ctt gtc tac tgg gca gat gcc tat
1627
Ile Thr Leu Asp Leu Val Ser Arg Leu Val Tyr Trp Ala Asp Ala Tyr
375 380 385

ctg gac tat att gaa gtg gtg gac tat gag ggc aag ggc cgc cag acc
1675
Leu Asp Tyr Ile Glu Val Val Asp Tyr Glu Gly Lys Gly Arg Gln Thr
390 395 400

atc atc cag ggc atc ctg att gag cac ctg tac ggc ctg act gtg ttt
1723
Ile Ile Gln Gly Ile Leu Ile Glu His Leu Tyr Gly Leu Thr Val Phe
405 410 415

gag aat tat ctc tat gcc acc aac tcg gac aat gcc aat gcc cag cag
1771
Glu Asn Tyr Leu Tyr Ala Thr Asn Ser Asp Asn Ala Asn Ala Gln Gln
420 425 430 435

aag acg agt gtg atc cgt gtg aac cgc ttt aac agc acc gag tac cag
1819
Lys Thr Ser Val Ile Arg Val Asn Arg Phe Asn Ser Thr Glu Tyr Gln
440 445 450

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1867
Val Val Thr Arg Val Asp Lys Gly Gly Ala Leu His Ile Tyr His Gln
455 460 465

agg cgt cag ccc cga gtg agg agc cat gcc tgt gaa aac gac cag tat
1915
Arg Arg Gln Pro Arg Val Arg Ser His Ala Cys Glu Asn Asp Gln Tyr
470 475 480

ggg aag ccg ggt ggc tgc tct gac atc tgc ctg ctg gcc aac agc cac
1963
Gly Lys Pro Gly Gly Cys Ser Asp Ile Cys Leu Leu Ala Asn Ser His
485 490 495

aag gcg cgg acc tgc cgc tgc cgt tcc ggc ttc agc ctg ggc agt gac
2011
Lys Ala Arg Thr Cys Arg Cys Arg Ser Gly Phe Ser Leu Gly Ser Asp
500 505 510 515

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ggg aag tca tgc aag aag ccg gag cat gag ctg ttc ctc gtg tat ggc
2059
Gly Lys Ser Cys Lys Lys Pro Glu His Glu Leu Phe Leu Val Tyr Gly
                520                 525                 530

aag ggc cgg cca ggc atc atc cgg ggc atg gat atg ggg gcc aag gtc
2107
Lys Gly Arg Pro Gly Ile Ile Arg Gly Met Asp Met Gly Ala Lys Val
            535                 540                 545

ccg gat gag cac atg atc ccc att gaa aac ctc atg aac ccc cga gcc
2155
Pro Asp Glu His Met Ile Pro Ile Glu Asn Leu Met Asn Pro Arg Ala
        550                 555                 560

ctg gac ttc cac gct gag acc ggc ttc atc tac ttt gcc gac acc acc
2203
Leu Asp Phe His Ala Glu Thr Gly Phe Ile Tyr Phe Ala Asp Thr Thr
    565                 570                 575

agc tac ctc att ggc cgc cag aag att gat ggc act gag cgg gag acc
2251
Ser Tyr Leu Ile Gly Arg Gln Lys Ile Asp Gly Thr Glu Arg Glu Thr
580                 585                 590                 595

atc ctg aag gac ggc atc cac aat gtg gag ggt gtg gcc gtg gac tgg
2299
Ile Leu Lys Asp Gly Ile His Asn Val Glu Gly Val Ala Val Asp Trp
                600                 605                 610

atg gga gac aat ctg tac tgg acg gac gat ggg ccc aaa aag aca atc
2347
Met Gly Asp Asn Leu Tyr Trp Thr Asp Asp Gly Pro Lys Lys Thr Ile
            615                 620                 625

agc gtg gcc agg ctg gag aaa gct gct cag acc cgc aag act tta atc
2395
Ser Val Ala Arg Leu Glu Lys Ala Ala Gln Thr Arg Lys Thr Leu Ile
        630                 635                 640

gag ggc aaa atg aca cac ccc agg gct att gtg gtg gat cca ctc aat
2443
Glu Gly Lys Met Thr His Pro Arg Ala Ile Val Val Asp Pro Leu Asn
    645                 650                 655

ggg tgg atg tac tgg aca gac tgg gag gag gac ccc aag gac agt cgg
2491
Gly Trp Met Tyr Trp Thr Asp Trp Glu Glu Asp Pro Lys Asp Ser Arg
660                 665                 670                 675

cgt ggg cgg ctg gag agg gcg tgg atg gat ggc tca cac cga gac atc
2539
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Arg Gly Arg Leu Glu Arg Ala Trp Met Asp Gly Ser His Arg Asp Ile
                680                 685                 690

ttt gtc acc tcc aag aca gtg ctt tgg ccc aat ggg cta agc ctg gac
2587
Phe Val Thr Ser Lys Thr Val Leu Trp Pro Asn Gly Leu Ser Leu Asp
            695                 700                 705

atc ccg gct ggg cgc ctc tac tgg gtg gat gcc ttc tac gac cgc atc
2635
Ile Pro Ala Gly Arg Leu Tyr Trp Val Asp Ala Phe Tyr Asp Arg Ile
        710                 715                 720

gag acg ata ctg ctc aat ggc aca gac cgg aag att gtg tat gaa ggt
2683
Glu Thr Ile Leu Leu Asn Gly Thr Asp Arg Lys Ile Val Tyr Glu Gly
    725                 730                 735

cct gag ctg aac cac gcc ttt ggc ctg tgt cac cat ggc aac tac ctc
2731
Pro Glu Leu Asn His Ala Phe Gly Leu Cys His His Gly Asn Tyr Leu
740                 745                 750                 755

ttc tgg act gag tat cgg agt ggc agt gtc tac cgc ttg gaa cgg ggt
2779
Phe Trp Thr Glu Tyr Arg Ser Gly Ser Val Tyr Arg Leu Glu Arg Gly
                760                 765                 770

gta gga ggc gca ccc ccc act gtg acc ctt ctg cgc agt gag cgg ccc
2827
Val Gly Gly Ala Pro Pro Thr Val Thr Leu Leu Arg Ser Glu Arg Pro
            775                 780                 785

ccc atc ttt gag atc cga atg tat gat gcc cag cag cag caa gtt ggc
2875
Pro Ile Phe Glu Ile Arg Met Tyr Asp Ala Gln Gln Gln Gln Val Gly
        790                 795                 800

acc aac aaa tgc cgg gtg aac aat ggc ggc tgc agc agc ctg tgc ttg
2923
Thr Asn Lys Cys Arg Val Asn Asn Gly Gly Cys Ser Ser Leu Cys Leu
    805                 810                 815

gcc acc cct ggg agc cgc cag tgc gcc tgt gct gag gac cag gtg ttg
2971
Ala Thr Pro Gly Ser Arg Gln Cys Ala Cys Ala Glu Asp Gln Val Leu
820                 825                 830                 835

gac gca gac ggc gtc act tgc ttg gcg aac cca tcc tac gtg cct cca
3019
Asp Ala Asp Gly Val Thr Cys Leu Ala Asn Pro Ser Tyr Val Pro Pro
                840                 845                 850
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ccc cag tgc cag cca ggc gag ttt gcc tgt gcc aac agc cgc tgc atc
3067
Pro Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser Arg Cys Ile
855 860 865

cag gag cgc tgg aag tgt gac gga gac aac gat tgc ctg gac aac agt
3115
Gln Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser
870 875 880

gat gag gcc cca gcc ctc tgc cat cag cac acc tgc ccc tcg gac cga
3163
Asp Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro Ser Asp Arg
885 890 895

ttc aag tgc gag aac aac cgg tgc atc ccc aac cgc tgg ctc tgc gac
3211
Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp
900 905 910 915

ggg gac aat gac tgt ggg aac agt gaa gat gag tcc aat gcc act tgt
3259
Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys
920 925 930

tca gcc cgc acc tgc ccc ccc aac cag ttc tcc tgt gcc agt ggc cgc
3307
Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg
935 940 945

tgc atc ccc atc tcc tgg acg tgt gat ctg gat gac gac tgt ggg gac
3355
Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp
950 955 960

cgc tct gat gag tct gct tcg tgt gcc tat ccc acc tgc ttc ccc ctg
3403
Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu
965 970 975

act cag ttt acc tgc aac aat ggc aga tgt atc aac atc aac tgg aga
3451
Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg
980 985 990 995

tgc gac aat gac aat gac tgt ggg gac aac agt gac gaa gcc ggc tgc
3499
Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys
1000 1005 1010

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Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys
1015 1020 1025

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Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr
1030 1035 1040

agt gat gag aca cac gcc aac tgc acc aac cag gcc acg agg ccc cct 3643
Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro
1045 1050 1055

ggt ggc tgc cac act gat gag ttc cag tgc cgg ctg gat gga cta tgc 3691
Gly Gly Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys
1060 1065 1070 1075

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Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr Asp Cys Met Asp Ser
1080 1085 1090

agc gat gag aag agc tgt gag gga gtg acc cac gtc tgc gat ccc agt 3787
Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His Val Cys Asp Pro Ser
1095 1100 1105

gtc aag ttt ggc tgc aag gac tca gct cgg tgc atc agc aaa gcg tgg 3835
Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp
1110 1115 1120

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Val Cys Asp Gly Asp Asn Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn
1125 1130 1135

tgc gag tcc ctg gcc tgc agg cca ccc tcg cac cct tgt gcc aac aac 3931
Cys Glu Ser Leu Ala Cys Arg Pro Pro Ser His Pro Cys Ala Asn Asn
1140 1145 1150 1155

acc tca gtc tgc ctg ccc cct gac aag ctg tgt gat ggc aac gac gac 3979
Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys Asp Gly Asn Asp Asp
1160 1165 1170

tgt ggc gac ggc tca gat gag ggc gag ctc tgc gac cag tgc tct ctg 4027

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Cys Gly Asp Gly Ser Asp Glu Gly Glu Leu Cys Asp Gln Cys Ser Leu
            1175                1180                1185

aat aac ggt ggc tgc agc cac aac tgc tca gtg gca cct ggc gaa ggc
4075
Asn Asn Gly Gly Cys Ser His Asn Cys Ser Val Ala Pro Gly Glu Gly
        1190                1195                1200

att gtg tgt tcc tgc cct ctg ggc atg gag ctg ggg ccc gac aac cac
4123
Ile Val Cys Ser Cys Pro Leu Gly Met Glu Leu Gly Pro Asp Asn His
    1205                1210                1215

acc tgc cag atc cag agc tac tgt gcc aag cat ctc aaa tgc agc caa
4171
Thr Cys Gln Ile Gln Ser Tyr Cys Ala Lys His Leu Lys Cys Ser Gln
1220                1225                1230                1235

aag tgc gac cag aac aag ttc agc gtg aag tgc tcc tgc tac gag ggc
4219
Lys Cys Asp Gln Asn Lys Phe Ser Val Lys Cys Ser Cys Tyr Glu Gly
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tgg gtc ctg gaa cct gac ggc gag agc tgc cgc agc ctg gac ccc ttc
4267
Trp Val Leu Glu Pro Asp Gly Glu Ser Cys Arg Ser Leu Asp Pro Phe
            1255                1260                1265

aag ccg ttc atc att ttc tcc aac cgc cat gaa atc cgg cgc atc gat
4315
Lys Pro Phe Ile Ile Phe Ser Asn Arg His Glu Ile Arg Arg Ile Asp
        1270                1275                1280

ctt cac aaa gga gac tac agc gtc ctg gtg ccc ggc ctg cgc aac acc
4363
Leu His Lys Gly Asp Tyr Ser Val Leu Val Pro Gly Leu Arg Asn Thr
    1285                1290                1295

atc gcc ctg gac ttc cac ctc agc cag agc gcc ctc tac tgg acc gac
4411
Ile Ala Leu Asp Phe His Leu Ser Gln Ser Ala Leu Tyr Trp Thr Asp
1300                1305                1310                1315

gtg gtg gag gac aag atc tac cgc ggg aag ctg ctg gac aac gga gcc
4459
Val Val Glu Asp Lys Ile Tyr Arg Gly Lys Leu Leu Asp Asn Gly Ala
                1320                1325                1330

ctg act agt ttc gag gtg gtg att cag tat ggc ctg gcc aca ccc gag
4507
Leu Thr Ser Phe Glu Val Val Ile Gln Tyr Gly Leu Ala Thr Pro Glu
            1335                1340                1345
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ggc ctg gct gta gac tgg att gca ggc aac atc tac tgg gtg gag agt
4555
Gly Leu Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Val Glu Ser
        1350                1355                1360

aac ctg gat cag atc gag gtg gcc aag ctg gat ggg acc ctc cgg acc
4603
Asn Leu Asp Gln Ile Glu Val Ala Lys Leu Asp Gly Thr Leu Arg Thr
    1365                1370                1375

acc ctg ctg gcc ggt gac att gag cac cca agg gca atc gca ctg gat
4651
Thr Leu Leu Ala Gly Asp Ile Glu His Pro Arg Ala Ile Ala Leu Asp
1380                1385                1390                1395

ccc cgg gat ggg atc ctg ttt tgg aca gac tgg gat gcc agc ctg ccc
4699
Pro Arg Asp Gly Ile Leu Phe Trp Thr Asp Trp Asp Ala Ser Leu Pro
                1400                1405                1410

cgc att gag gca gcc tcc atg agt ggg gct ggg cgc cgc acc gtg cac
4747
Arg Ile Glu Ala Ala Ser Met Ser Gly Ala Gly Arg Arg Thr Val His
            1415                1420                1425

cgg gag acc ggc tct ggg ggc tgg ccc aac ggg ctc acc gtg gac tac
4795
Arg Glu Thr Gly Ser Gly Gly Trp Pro Asn Gly Leu Thr Val Asp Tyr
        1430                1435                1440

ctg gag aag cgc atc ctt tgg att gac gcc agg tca gat gcc att tac
4843
Leu Glu Lys Arg Ile Leu Trp Ile Asp Ala Arg Ser Asp Ala Ile Tyr
    1445                1450                1455

tca gcc cgt tac gac ggc tct ggc cac atg gag gtg ctt cgg gga cac
4891
Ser Ala Arg Tyr Asp Gly Ser Gly His Met Glu Val Leu Arg Gly His
1460                1465                1470                1475

gag ttc ctg tcg cac ccg ttt gca gtg acg ctg tac ggg ggg gag gtc
4939
Glu Phe Leu Ser His Pro Phe Ala Val Thr Leu Tyr Gly Gly Glu Val
                1480                1485                1490

tac tgg act gac tgg cga aca aac aca ctg gct aag gcc aac aag tgg
4987
Tyr Trp Thr Asp Trp Arg Thr Asn Thr Leu Ala Lys Ala Asn Lys Trp
            1495                1500                1505
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acc ggc cac aat gtc acc gtg gta cag agg acc aac acc cag ccc ttt
5035
Thr Gly His Asn Val Thr Val Val Gln Arg Thr Asn Thr Gln Pro Phe
1510 1515 1520

gac ctg cag gtg tac cac ccc tcc cgc cag ccc atg gct ccc aat ccc
5083
Asp Leu Gln Val Tyr His Pro Ser Arg Gln Pro Met Ala Pro Asn Pro
1525 1530 1535

tgt gag gcc aat ggg ggc cag ggc ccc tgc tcc cac ctg tgt ctc atc
5131
Cys Glu Ala Asn Gly Gly Gln Gly Pro Cys Ser His Leu Cys Leu Ile
1540 1545 1550 1555

aac tac aac cgg acc gtg tcc tgc gcc tgc ccc cac ctc atg aag ctc
5179
Asn Tyr Asn Arg Thr Val Ser Cys Ala Cys Pro His Leu Met Lys Leu
1560 1565 1570

cac aag gac aac acc acc tgc tat gag ttt aag aag ttc ctg ctg tac
5227
His Lys Asp Asn Thr Thr Cys Tyr Glu Phe Lys Lys Phe Leu Leu Tyr
1575 1580 1585

gca cgt cag atg gag atc cga ggt gtg gac ctg gat gct ccc tac tac
5275
Ala Arg Gln Met Glu Ile Arg Gly Val Asp Leu Asp Ala Pro Tyr Tyr
1590 1595 1600

aac tac atc atc tcc ttc acg gtg ccc gac atc gac aac gtc aca gtg
5323
Asn Tyr Ile Ile Ser Phe Thr Val Pro Asp Ile Asp Asn Val Thr Val
1605 1610 1615

cta gac tac gat gcc cgc gag cag cgt gtg tac tgg tct gac gtg cgg
5371
Leu Asp Tyr Asp Ala Arg Glu Gln Arg Val Tyr Trp Ser Asp Val Arg
1620 1625 1630 1635

aca cag gcc atc aag cgg gcc ttc atc aac ggc aca ggc gtg gag aca
5419
Thr Gln Ala Ile Lys Arg Ala Phe Ile Asn Gly Thr Gly Val Glu Thr
1640 1645 1650

gtc gtc tct gca gac ttg cca aat gcc cac ggg ctg gct gtg gac tgg
5467
Val Val Ser Ala Asp Leu Pro Asn Ala His Gly Leu Ala Val Asp Trp
1655 1660 1665

gtc tcc cga aac ctg ttc tgg aca agc tat gac acc aat aag aag cag
5515

Val Ser Arg Asn Leu Phe Trp Thr Ser Tyr Asp Thr Asn Lys Lys Gln
1670 1675 1680

atc aat gtg gcc cgg ctg gat ggc tcc ttc aag aac gca gtg gtg cag
5563
Ile Asn Val Ala Arg Leu Asp Gly Ser Phe Lys Asn Ala Val Val Gln
1685 1690 1695

ggc ctg gag cag ccc cat ggc ctt gtc gtc cac cct ctg cgt ggg aag
5611
Gly Leu Glu Gln Pro His Gly Leu Val Val His Pro Leu Arg Gly Lys
1700 1705 1710 1715

ctc tac tgg acc gat ggt gac aac atc agc atg gcc aac atg gat ggc
5659
Leu Tyr Trp Thr Asp Gly Asp Asn Ile Ser Met Ala Asn Met Asp Gly
1720 1725 1730

agc aat cgc acc ctg ctc ttc agt ggc cag aag ggc ccc gtg ggc ctg
5707
Ser Asn Arg Thr Leu Leu Phe Ser Gly Gln Lys Gly Pro Val Gly Leu
1735 1740 1745

gct att gac ttc cct gaa agc aaa ctc tac tgg atc agc tcc ggg aac
5755
Ala Ile Asp Phe Pro Glu Ser Lys Leu Tyr Trp Ile Ser Ser Gly Asn
1750 1755 1760

cat acc atc aac cgc tgc aac ctg gat ggg agt ggg ctg gag gtc atc
5803
His Thr Ile Asn Arg Cys Asn Leu Asp Gly Ser Gly Leu Glu Val Ile
1765 1770 1775

gat gcc atg cgg agc cag ctg ggc aag gcc acc gcc ctg gcc atc atg
5851
Asp Ala Met Arg Ser Gln Leu Gly Lys Ala Thr Ala Leu Ala Ile Met
1780 1785 1790 1795

ggg gac aag ctg tgg tgg gct gat cag gtg tcg gaa aag atg ggc aca
5899
Gly Asp Lys Leu Trp Trp Ala Asp Gln Val Ser Glu Lys Met Gly Thr
1800 1805 1810

tgc agc aag gct gac ggc tcg ggc tcc gtg gtc ctt cgg aac agc acc
5947
Cys Ser Lys Ala Asp Gly Ser Gly Ser Val Val Leu Arg Asn Ser Thr
1815 1820 1825

acc ctg gtg atg cac atg aag gtc tat gac gag agc atc cag ctg gac
5995
Thr Leu Val Met His Met Lys Val Tyr Asp Glu Ser Ile Gln Leu Asp
1830 1835 1840

cat aag ggc acc aac ccc tgc agt gtc aac aac ggt gac tgc tcc cag
6043
His Lys Gly Thr Asn Pro Cys Ser Val Asn Asn Gly Asp Cys Ser Gln
1845 1850 1855

ctc tgc ctg ccc acg tca gag acg acc cgc tcc tgc atg tgc aca gcc
6091
Leu Cys Leu Pro Thr Ser Glu Thr Thr Arg Ser Cys Met Cys Thr Ala
1860 1865 1870 1875

ggc tat agc ctc cgg agt ggc cag cag gcc tgc gag ggc gta ggt tcc
6139
Gly Tyr Ser Leu Arg Ser Gly Gln Gln Ala Cys Glu Gly Val Gly Ser
1880 1885 1890

ttt ctc ctg tac tct gtg cat gag gga atc agg gga att ccc ctg gat
6187
Phe Leu Leu Tyr Ser Val His Glu Gly Ile Arg Gly Ile Pro Leu Asp
1895 1900 1905

ccc aat gac aag tca gat gcc ctg gtc cca gtg tcc ggg acc tcg ctg
6235
Pro Asn Asp Lys Ser Asp Ala Leu Val Pro Val Ser Gly Thr Ser Leu
1910 1915 1920

gct gtc ggc atc gac ttc cac gct gaa aat gac acc atc tac tgg gtg
6283
Ala Val Gly Ile Asp Phe His Ala Glu Asn Asp Thr Ile Tyr Trp Val
1925 1930 1935

gac atg ggc ctg agc acg atc agc cgg gcc aag cgg gac cag acg tgg
6331
Asp Met Gly Leu Ser Thr Ile Ser Arg Ala Lys Arg Asp Gln Thr Trp
1940 1945 1950 1955

cgt gaa gac gtg gtg acc aat ggc att ggc cgt gtg gag ggc att gca
6379
Arg Glu Asp Val Val Thr Asn Gly Ile Gly Arg Val Glu Gly Ile Ala
1960 1965 1970

gtg gac tgg atc gca ggc aac atc tac tgg aca gac cag ggc ttt gat
6427
Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Thr Asp Gln Gly Phe Asp
1975 1980 1985

gtc atc gag gtc gcc cgg ctc aat ggc tcc ttc cgc tac gtg gtg atc
6475
Val Ile Glu Val Ala Arg Leu Asn Gly Ser Phe Arg Tyr Val Val Ile
1990 1995 2000

tcc cag ggt cta gac aag ccc cgg gcc atc acc gtc cac ccg gag aaa
6523
Ser Gln Gly Leu Asp Lys Pro Arg Ala Ile Thr Val His Pro Glu Lys
2005 2010 2015

ggg tac ttg ttc tgg act gag tgg ggt cag tat ccg cgt att gag cgg
6571
Gly Tyr Leu Phe Trp Thr Glu Trp Gly Gln Tyr Pro Arg Ile Glu Arg
2020 2025 2030 2035

tct cgg cta gat ggc acg gag cgt gtg gtg ctg gtc aac gtc agc atc
6619
Ser Arg Leu Asp Gly Thr Glu Arg Val Val Leu Val Asn Val Ser Ile
2040 2045 2050

agc tgg ccc aac ggc atc tca gtg gac tac cag gat ggg aag ctg tac
6667
Ser Trp Pro Asn Gly Ile Ser Val Asp Tyr Gln Asp Gly Lys Leu Tyr
2055 2060 2065

tgg tgc gat gca cgg aca gac aag att gaa cgg atc gac ctg gag aca
6715
Trp Cys Asp Ala Arg Thr Asp Lys Ile Glu Arg Ile Asp Leu Glu Thr
2070 2075 2080

ggt gag aac cgc gag gtg gtt ctg tcc agc aac aac atg gac atg ttt
6763
Gly Glu Asn Arg Glu Val Val Leu Ser Ser Asn Asn Met Asp Met Phe
2085 2090 2095

tca gtg tct gtg ttt gag gat ttc atc tac tgg agt gac agg act cat
6811
Ser Val Ser Val Phe Glu Asp Phe Ile Tyr Trp Ser Asp Arg Thr His
2100 2105 2110 2115

gcc aac ggc tct atc aag cgc ggg agc aaa gac aat gcc aca gac tcc
6859
Ala Asn Gly Ser Ile Lys Arg Gly Ser Lys Asp Asn Ala Thr Asp Ser
2120 2125 2130

gtg ccc ctg cga acc ggc atc ggc gtc cag ctt aaa gac atc aaa gtc
6907
Val Pro Leu Arg Thr Gly Ile Gly Val Gln Leu Lys Asp Ile Lys Val
2135 2140 2145

ttc aac cgg gac cgg cag aaa ggc acc aac gtg tgc gcg gtg gcc aat
6955
Phe Asn Arg Asp Arg Gln Lys Gly Thr Asn Val Cys Ala Val Ala Asn
2150 2155 2160

ggc ggg tgc cag cag ctg tgc ctg tac cgg ggc cgt ggg cag cgg gcc
7003

Gly Gly Cys Gln Gln Leu Cys Leu Tyr Arg Gly Arg Gly Gln Arg Ala
2165 2170 2175

tgc gcc tgt gcc cac ggg atg ctg gct gaa gac gga gca tcg tgc cgc
7051
Cys Ala Cys Ala His Gly Met Leu Ala Glu Asp Gly Ala Ser Cys Arg
2180 2185 2190 2195

gag tat gcc ggc tac ctg ctc tac tca gag cgc acc att ctc aag agt
7099
Glu Tyr Ala Gly Tyr Leu Leu Tyr Ser Glu Arg Thr Ile Leu Lys Ser
2200 2205 2210

atc cac ctg tcg gat gag cgc aac ctc aat gcg ccc gtg cag ccc ttc
7147
Ile His Leu Ser Asp Glu Arg Asn Leu Asn Ala Pro Val Gln Pro Phe
2215 2220 2225

gag gac cct gag cac atg aag aac gtc atc gcc ctg gcc ttt gac tac
7195
Glu Asp Pro Glu His Met Lys Asn Val Ile Ala Leu Ala Phe Asp Tyr
2230 2235 2240

cgg gca ggc acc tct ccg ggc acc ccc aat cgc atc ttc ttc agc gac
7243
Arg Ala Gly Thr Ser Pro Gly Thr Pro Asn Arg Ile Phe Phe Ser Asp
2245 2250 2255

atc cac ttt ggg aac atc caa cag atc aac gac gat ggc tcc agg agg
7291
Ile His Phe Gly Asn Ile Gln Gln Ile Asn Asp Asp Gly Ser Arg Arg
2260 2265 2270 2275

atc acc att gtg gaa aac gtg ggc tcc gtg gaa ggc ctg gcc tat cac
7339
Ile Thr Ile Val Glu Asn Val Gly Ser Val Glu Gly Leu Ala Tyr His
2280 2285 2290

cgt ggc tgg gac act ctc tat tgg aca agc tac acg aca tcc acc atc
7387
Arg Gly Trp Asp Thr Leu Tyr Trp Thr Ser Tyr Thr Thr Ser Thr Ile
2295 2300 2305

acg cgc cac aca gtg gac cag acc cgc cca ggg gcc ttc gag cgt gag
7435
Thr Arg His Thr Val Asp Gln Thr Arg Pro Gly Ala Phe Glu Arg Glu
2310 2315 2320

acc gtc atc act atg tct gga gat gac cac cca cgg gcc ttc gtt ttg
7483
Thr Val Ile Thr Met Ser Gly Asp Asp His Pro Arg Ala Phe Val Leu
2325 2330 2335

gac gag tgc cag aac ctc atg ttc tgg acc aac tgg aat gag cag cat
7531
Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp Asn Glu Gln His
2340 2345 2350 2355

ccc agc atc atg cgg gcg gcg ctc tcg gga gcc aat gtc ctg acc ctt
7579
Pro Ser Ile Met Arg Ala Ala Leu Ser Gly Ala Asn Val Leu Thr Leu
2360 2365 2370

atc gag aag gac atc cgt acc ccc aat ggc ctg gcc atc gac cac cgt
7627
Ile Glu Lys Asp Ile Arg Thr Pro Asn Gly Leu Ala Ile Asp His Arg
2375 2380 2385

gcc gag aag ctc tac ttc tct gac gcc acc ctg gac aag atc gag cgg
7675
Ala Glu Lys Leu Tyr Phe Ser Asp Ala Thr Leu Asp Lys Ile Glu Arg
2390 2395 2400

tgc gag tat gac ggc tcc cac cgc tat gtg atc cta aag tca gag cct
7723
Cys Glu Tyr Asp Gly Ser His Arg Tyr Val Ile Leu Lys Ser Glu Pro
2405 2410 2415

gtc cac ccc ttc ggg ctg gcc gtg tat ggg gag cac att ttc tgg act
7771
Val His Pro Phe Gly Leu Ala Val Tyr Gly Glu His Ile Phe Trp Thr
2420 2425 2430 2435

gac tgg gtg cgg cgg gca gtg cag cgg gcc aac aag cac gtg ggc agc
7819
Asp Trp Val Arg Arg Ala Val Gln Arg Ala Asn Lys His Val Gly Ser
2440 2445 2450

aac atg aag ctg ctg cgc gtg gac atc ccc cag cag ccc atg ggc atc
7867
Asn Met Lys Leu Leu Arg Val Asp Ile Pro Gln Gln Pro Met Gly Ile
2455 2460 2465

atc gcc gtg gcc aac gac acc aac agc tgt gaa ctc tct cca tgc cga
7915
Ile Ala Val Ala Asn Asp Thr Asn Ser Cys Glu Leu Ser Pro Cys Arg
2470 2475 2480

atc aac aac ggt ggc tgc cag gac ctg tgt ctg ctc act cac cag ggc
7963
Ile Asn Asn Gly Gly Cys Gln Asp Leu Cys Leu Leu Thr His Gln Gly
2485 2490 2495

cat gtc aac tgc tca tgc cga ggg ggc cga atc ctc cag gat gac ctc
8011
His Val Asn Cys Ser Cys Arg Gly Gly Arg Ile Leu Gln Asp Asp Leu
2500 2505 2510 2515

acc tgc cga gcg gtg aat tcc tct tgc cga gca caa gat gag ttt gag
8059
Thr Cys Arg Ala Val Asn Ser Ser Cys Arg Ala Gln Asp Glu Phe Glu
2520 2525 2530

tgt gcc aat ggc gag tgc atc aac ttc agc ctg acc tgc gac ggc gtc
8107
Cys Ala Asn Gly Glu Cys Ile Asn Phe Ser Leu Thr Cys Asp Gly Val
2535 2540 2545

ccc cac tgc aag gac aag tcc gat gag aag cca tcc tac tgc aac tcc
8155
Pro His Cys Lys Asp Lys Ser Asp Glu Lys Pro Ser Tyr Cys Asn Ser
2550 2555 2560

cgc cgc tgc aag aag act ttc cgg cag tgc agc aat ggg cgc tgt gtg
8203
Arg Arg Cys Lys Lys Thr Phe Arg Gln Cys Ser Asn Gly Arg Cys Val
2565 2570 2575

tcc aac atg ctg tgg tgc aac ggg gcc gac gac tgt ggg gat ggc tct
8251
Ser Asn Met Leu Trp Cys Asn Gly Ala Asp Asp Cys Gly Asp Gly Ser
2580 2585 2590 2595

gac gag atc cct tgc aac aag aca gcc tgt ggt gtg ggc gag ttc cgc
8299
Asp Glu Ile Pro Cys Asn Lys Thr Ala Cys Gly Val Gly Glu Phe Arg
2600 2605 2610

tgc cgg gac ggg acc tgc atc ggg aac tcc agc cgc tgc aac cag ttt
8347
Cys Arg Asp Gly Thr Cys Ile Gly Asn Ser Ser Arg Cys Asn Gln Phe
2615 2620 2625

gtg gat tgt gag gac gcc tca gat gag atg aac tgc agt gcc acc gac
8395
Val Asp Cys Glu Asp Ala Ser Asp Glu Met Asn Cys Ser Ala Thr Asp
2630 2635 2640

tgc agc agc tac ttc cgc ctg ggc gtg aag ggc gtg ctc ttc cag ccc
8443
Cys Ser Ser Tyr Phe Arg Leu Gly Val Lys Gly Val Leu Phe Gln Pro
2645 2650 2655

tgc gag cgg acc tca ctc tgc tac gca ccc agc tgg gtg tgt gat ggc
8491

Cys Glu Arg Thr Ser Leu Cys Tyr Ala Pro Ser Trp Val Cys Asp Gly
2660 2665 2670 2675

gcc aat gac tgt ggg gac tac agt gat gag cgc gac tgc cca ggt gtg
8539
Ala Asn Asp Cys Gly Asp Tyr Ser Asp Glu Arg Asp Cys Pro Gly Val
2680 2685 2690

aaa cgc ccc aga tgc cct ctg aat tac ttc gcc tgc cct agt ggg cgc
8587
Lys Arg Pro Arg Cys Pro Leu Asn Tyr Phe Ala Cys Pro Ser Gly Arg
2695 2700 2705

tgc atc ccc atg agc tgg acg tgt gac aaa gag gat gac tgt gaa cat
8635
Cys Ile Pro Met Ser Trp Thr Cys Asp Lys Glu Asp Asp Cys Glu His
2710 2715 2720

ggc gag gac gag acc cac tgc aac aag ttc tgc tca gag gcc cag ttt
8683
Gly Glu Asp Glu Thr His Cys Asn Lys Phe Cys Ser Glu Ala Gln Phe
2725 2730 2735

gag tgc cag aac cat cgc tgc atc tcc aag cag tgg ctg tgt gac ggc
8731
Glu Cys Gln Asn His Arg Cys Ile Ser Lys Gln Trp Leu Cys Asp Gly
2740 2745 2750 2755

agc gat gac tgt ggg gat ggc tca gac gag gct gct cac tgt gaa ggc
8779
Ser Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ala His Cys Glu Gly
2760 2765 2770

aag acg tgc ggc ccc tcc tcc ttc tcc tgc cct ggc acc cac gtg tgc
8827
Lys Thr Cys Gly Pro Ser Ser Phe Ser Cys Pro Gly Thr His Val Cys
2775 2780 2785

gtc ccc gag cgc tgg ctc tgt gac ggt gac aaa gac tgt gct gat ggt
8875
Val Pro Glu Arg Trp Leu Cys Asp Gly Asp Lys Asp Cys Ala Asp Gly
2790 2795 2800

gca gac gag agc atc gca gct ggt tgc ttg tac aac agc act tgt gac
8923
Ala Asp Glu Ser Ile Ala Ala Gly Cys Leu Tyr Asn Ser Thr Cys Asp
2805 2810 2815

gac cgt gag ttc atg tgc cag aac cgc cag tgc atc ccc aag cac ttc
8971
Asp Arg Glu Phe Met Cys Gln Asn Arg Gln Cys Ile Pro Lys His Phe
2820 2825 2830 2835

gtg tgt gac cac gac cgt gac tgt gca gat ggc tct gat gag tcc ccc
9019
Val Cys Asp His Asp Arg Asp Cys Ala Asp Gly Ser Asp Glu Ser Pro
2840 2845 2850

gag tgt gag tac ccg acc tgc ggc ccc agt gag ttc cgc tgt gcc aat
9067
Glu Cys Glu Tyr Pro Thr Cys Gly Pro Ser Glu Phe Arg Cys Ala Asn
2855 2860 2865

ggg cgc tgt ctg agc tcc cgc cag tgg gag tgt gat ggc gag aat gac
9115
Gly Arg Cys Leu Ser Ser Arg Gln Trp Glu Cys Asp Gly Glu Asn Asp
2870 2875 2880

tgc cac gac cag agt gac gag gct ccc aag aac cca cac tgc acc agc
9163
Cys His Asp Gln Ser Asp Glu Ala Pro Lys Asn Pro His Cys Thr Ser
2885 2890 2895

cca gag cac aag tgc aat gcc tcg tca cag ttc ctg tgc agc agt ggg
9211
Pro Glu His Lys Cys Asn Ala Ser Ser Gln Phe Leu Cys Ser Ser Gly
2900 2905 2910 2915

cgc tgt gtg gct gag gca ctg ctc tgc aac ggc cag gat gac tgt ggc
9259
Arg Cys Val Ala Glu Ala Leu Leu Cys Asn Gly Gln Asp Asp Cys Gly
2920 2925 2930

gac agc tcg gac gag cgt ggc tgc cac atc aat gag tgt ctc agc cgc
9307
Asp Ser Ser Asp Glu Arg Gly Cys His Ile Asn Glu Cys Leu Ser Arg
2935 2940 2945

aag ctc agt ggc tgc agc cag gac tgt gag gac ctc aag atc ggc ttc
9355
Lys Leu Ser Gly Cys Ser Gln Asp Cys Glu Asp Leu Lys Ile Gly Phe
2950 2955 2960

aag tgc cgc tgt cgc cct ggc ttc cgg ctg aag gat gac ggc cgg acg
9403
Lys Cys Arg Cys Arg Pro Gly Phe Arg Leu Lys Asp Asp Gly Arg Thr
2965 2970 2975

tgt gct gat gtg gac gag tgc agc acc acc ttc ccc tgc agc cag cgc
9451
Cys Ala Asp Val Asp Glu Cys Ser Thr Thr Phe Pro Cys Ser Gln Arg
2980 2985 2990 2995

tgc atc aac acc cat ggc agc tat aag tgt ctg tgt gtg gag ggc tat
9499
Cys Ile Asn Thr His Gly Ser Tyr Lys Cys Leu Cys Val Glu Gly Tyr
3000 3005 3010

gca ccc cgc ggc ggc gac ccc cac agc tgc aag gct gtg act gac gag
9547
Ala Pro Arg Gly Gly Asp Pro His Ser Cys Lys Ala Val Thr Asp Glu
3015 3020 3025

gaa ccg ttt ctg atc ttc gcc aac cgg tac tac ctg cgc aag ctc aac
9595
Glu Pro Phe Leu Ile Phe Ala Asn Arg Tyr Tyr Leu Arg Lys Leu Asn
3030 3035 3040

ctg gac ggg tcc aac tac acg tta ctt aag cag ggc ctg aac aac gcc
9643
Leu Asp Gly Ser Asn Tyr Thr Leu Leu Lys Gln Gly Leu Asn Asn Ala
3045 3050 3055

gtt gcc ttg gat ttt gac tac cga gag cag atg atc tac tgg aca gat
9691
Val Ala Leu Asp Phe Asp Tyr Arg Glu Gln Met Ile Tyr Trp Thr Asp
3060 3065 3070 3075

gtg acc acc cag ggc agc atg atc cga agg atg cac ctt aac ggg agc
9739
Val Thr Thr Gln Gly Ser Met Ile Arg Arg Met His Leu Asn Gly Ser
3080 3085 3090

aat gtg cag gtc cta cac cgt aca ggc ctc agc aac ccc gat ggg ctg
9787
Asn Val Gln Val Leu His Arg Thr Gly Leu Ser Asn Pro Asp Gly Leu
3095 3100 3105

gct gtg gac tgg gtg ggt ggc aac ctg tac tgg tgc gac aaa ggc cgg
9835
Ala Val Asp Trp Val Gly Gly Asn Leu Tyr Trp Cys Asp Lys Gly Arg
3110 3115 3120

gac acc atc gag gtg tcc aag ctc aat ggg gcc tat cgg acg gtg ctg
9883
Asp Thr Ile Glu Val Ser Lys Leu Asn Gly Ala Tyr Arg Thr Val Leu
3125 3130 3135

gtc agc tct ggc ctc cgt gag ccc agg gct ctg gtg gtg gat gtg cag
9931
Val Ser Ser Gly Leu Arg Glu Pro Arg Ala Leu Val Val Asp Val Gln
3140 3145 3150 3155

aat ggg tac ctg tac tgg aca gac tgg ggt gac cat tca ctg atc ggc
9979

Asn Gly Tyr Leu Tyr Trp Thr Asp Trp Gly Asp His Ser Leu Ile Gly
3160 3165 3170

cgc atc ggc atg gat ggg tcc agc cgc agc gtc atc gtg gac acc aag
10027
Arg Ile Gly Met Asp Gly Ser Ser Arg Ser Val Ile Val Asp Thr Lys
3175 3180 3185

atc aca tgg ccc aat ggc ctg acg ctg gac tat gtc act gag cgc atc
10075
Ile Thr Trp Pro Asn Gly Leu Thr Leu Asp Tyr Val Thr Glu Arg Ile
3190 3195 3200

tac tgg gcc gac gcc cgc gag gac tac att gaa ttt gcc agc ctg gat
10123
Tyr Trp Ala Asp Ala Arg Glu Asp Tyr Ile Glu Phe Ala Ser Leu Asp
3205 3210 3215

ggc tcc aat cgc cac gtt gtg ctg agc cag gac atc ccg cac atc ttt
10171
Gly Ser Asn Arg His Val Val Leu Ser Gln Asp Ile Pro His Ile Phe
3220 3225 3230 3235

gca ctg acc ctg ttt gag gac tac gtc tac tgg acc gac tgg gaa aca
10219
Ala Leu Thr Leu Phe Glu Asp Tyr Val Tyr Trp Thr Asp Trp Glu Thr
3240 3245 3250

aag tcc att aac cga gcc cac aag acc acg ggc acc aac aaa acg ctc
10267
Lys Ser Ile Asn Arg Ala His Lys Thr Thr Gly Thr Asn Lys Thr Leu
3255 3260 3265

ctc atc agc acg ctg cac cgg ccc atg gac ctg cat gtc ttc cat gcc
10315
Leu Ile Ser Thr Leu His Arg Pro Met Asp Leu His Val Phe His Ala
3270 3275 3280

ctg cgc cag cca gac gtg ccc aat cac ccc tgc aag gtc aac aat ggt
10363
Leu Arg Gln Pro Asp Val Pro Asn His Pro Cys Lys Val Asn Asn Gly
3285 3290 3295

ggc tgc agc aac ctg tgc ctg ctg tcc ccc ggg gga ggg cac aaa tgt
10411
Gly Cys Ser Asn Leu Cys Leu Leu Ser Pro Gly Gly Gly His Lys Cys
3300 3305 3310 3315

gcc tgc ccc acc aac ttc tac ctg ggc agc gat ggg cgc acc tgt gtg
10459
Ala Cys Pro Thr Asn Phe Tyr Leu Gly Ser Asp Gly Arg Thr Cys Val
3320 3325 3330

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tcc aac tgc acg gct agc cag ttt gta tgc aag aac gac aag tgc atc
10507
Ser Asn Cys Thr Ala Ser Gln Phe Val Cys Lys Asn Asp Lys Cys Ile
            3335                3340                3345

ccc ttc tgg tgg aag tgt gac acc gag gac gac tgc ggg gac cac tca
10555
Pro Phe Trp Trp Lys Cys Asp Thr Glu Asp Asp Cys Gly Asp His Ser
        3350                3355                3360

gac gag ccc ccg gac tgc cct gag ttc aag tgc cgg ccc gga cag ttc
10603
Asp Glu Pro Pro Asp Cys Pro Glu Phe Lys Cys Arg Pro Gly Gln Phe
    3365                3370                3375

cag tgc tcc aca ggt atc tgc aca aac cct gcc ttc atc tgc gat ggc
10651
Gln Cys Ser Thr Gly Ile Cys Thr Asn Pro Ala Phe Ile Cys Asp Gly
3380                3385                3390                3395

gac aat gac tgc cag gac aac agt gac gag gcc aac tgt gac atc cac
10699
Asp Asn Asp Cys Gln Asp Asn Ser Asp Glu Ala Asn Cys Asp Ile His
                3400                3405                3410

gtc tgc ttg ccc agt cag ttc aaa tgc acc aac acc aac cgc tgt att
10747
Val Cys Leu Pro Ser Gln Phe Lys Cys Thr Asn Thr Asn Arg Cys Ile
            3415                3420                3425

ccc ggc atc ttc cgc tgc aat ggg cag gac aac tgc gga gat ggg gag
10795
Pro Gly Ile Phe Arg Cys Asn Gly Gln Asp Asn Cys Gly Asp Gly Glu
        3430                3435                3440

gat gag agg gac tgc ccc gag gtg acc tgc gcc ccc aac cag ttc cag
10843
Asp Glu Arg Asp Cys Pro Glu Val Thr Cys Ala Pro Asn Gln Phe Gln
    3445                3450                3455

tgc tcc att acc aaa cgg tgc atc ccc cgg gtc tgg gtc tgc gac cgg
10891
Cys Ser Ile Thr Lys Arg Cys Ile Pro Arg Val Trp Val Cys Asp Arg
3460                3465                3470                3475

gac aat gac tgt gtg gat ggc agt gat gag ccc gcc aac tgc acc cag
10939
Asp Asn Asp Cys Val Asp Gly Ser Asp Glu Pro Ala Asn Cys Thr Gln
                3480                3485                3490
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atg acc tgt ggt gtg gac gag ttc cgc tgc aag gat tcg ggc cgc tgc
10987
Met Thr Cys Gly Val Asp Glu Phe Arg Cys Lys Asp Ser Gly Arg Cys
3495 3500 3505

atc cca gcg cgt tgg aag tgt gac gga gag gat gac tgt ggg gat ggc
11035
Ile Pro Ala Arg Trp Lys Cys Asp Gly Glu Asp Asp Cys Gly Asp Gly
3510 3515 3520

tcg gat gag ccc aag gaa gag tgt gat gaa cgc acc tgt gag cca tac
11083
Ser Asp Glu Pro Lys Glu Glu Cys Asp Glu Arg Thr Cys Glu Pro Tyr
3525 3530 3535

cag ttc cgc tgc aag aac aac cgc tgc gtg ccc ggc cgc tgg cag tgc
11131
Gln Phe Arg Cys Lys Asn Asn Arg Cys Val Pro Gly Arg Trp Gln Cys
3540 3545 3550 3555

gac tac gac aac gat tgc ggt gac aac tcc gat gaa gag agc tgc acc
11179
Asp Tyr Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Glu Ser Cys Thr
3560 3565 3570

cct cgg ccc tgc tcc gag agt gag ttc tcc tgt gcc aac ggc cgc tgc
11227
Pro Arg Pro Cys Ser Glu Ser Glu Phe Ser Cys Ala Asn Gly Arg Cys
3575 3580 3585

atc gcg ggg cgc tgg aaa tgc gat gga gac cac gac tgc gcg gac ggc
11275
Ile Ala Gly Arg Trp Lys Cys Asp Gly Asp His Asp Cys Ala Asp Gly
3590 3595 3600

tcg gac gag aaa gac tgc acc ccc cgc tgt gac atg gac cag ttc cag
11323
Ser Asp Glu Lys Asp Cys Thr Pro Arg Cys Asp Met Asp Gln Phe Gln
3605 3610 3615

tgc aag agc ggc cac tgc atc ccc ctg cgc tgg cgc tgt gac gca gac
11371
Cys Lys Ser Gly His Cys Ile Pro Leu Arg Trp Arg Cys Asp Ala Asp
3620 3625 3630 3635

gcc gac tgc atg gac ggc agc gac gag gag gcc tgc ggc act ggc gtg
11419
Ala Asp Cys Met Asp Gly Ser Asp Glu Glu Ala Cys Gly Thr Gly Val
3640 3645 3650

cgg acc tgc ccc ctg gac gag ttc cag tgc aac aac acc ttg tgc aag
11467

Arg Thr Cys Pro Leu Asp Glu Phe Gln Cys Asn Asn Thr Leu Cys Lys
3655 3660 3665

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Pro Gln Ser Lys Ala Gln Arg Cys Gln Pro Asn Glu His Asn Cys Leu
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Gly Asn Cys Ser Arg Leu Gly Cys Gln His His Cys Val Pro Thr Leu
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Asp Ser Arg Arg Gly Arg Leu Glu Arg Ala Trp Met Asp Gly Ser His
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Ser Leu Asp Ile Pro Ala Gly Arg Leu Tyr Trp Val Asp Ala Phe Tyr
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Ile Glu Arg Ser Arg Leu Asp Gly Thr Glu Arg Val Val Leu Val Asn
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Val Ser Ile Ser Trp Pro Asn Gly Ile Ser Val Asp Tyr Gln Asp Gly
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    2050                2055                2060
Lys Leu Tyr Trp Cys Asp Ala Arg Thr Asp Lys Ile Glu Arg Ile Asp
2065                2070                2075                2080
Leu Glu Thr Gly Glu Asn Arg Glu Val Val Leu Ser Ser Asn Asn Met
                2085                2090                2095
Asp Met Phe Ser Val Ser Val Phe Glu Asp Phe Ile Tyr Trp Ser Asp
            2100                2105                2110
Arg Thr His Ala Asn Gly Ser Ile Lys Arg Gly Ser Lys Asp Asn Ala
        2115                2120                2125
Thr Asp Ser Val Pro Leu Arg Thr Gly Ile Gly Val Gln Leu Lys Asp
    2130                2135                2140
Ile Lys Val Phe Asn Arg Asp Arg Gln Lys Gly Thr Asn Val Cys Ala
2145                2150                2155                2160
Val Ala Asn Gly Gly Cys Gln Gln Leu Cys Leu Tyr Arg Gly Arg Gly
                2165                2170                2175
Gln Arg Ala Cys Ala Cys Ala His Gly Met Leu Ala Glu Asp Gly Ala
            2180                2185                2190
Ser Cys Arg Glu Tyr Ala Gly Tyr Leu Leu Tyr Ser Glu Arg Thr Ile
        2195                2200                2205
Leu Lys Ser Ile His Leu Ser Asp Glu Arg Asn Leu Asn Ala Pro Val
    2210                2215                2220
Gln Pro Phe Glu Asp Pro Glu His Met Lys Asn Val Ile Ala Leu Ala
2225                2230                2235                2240
Phe Asp Tyr Arg Ala Gly Thr Ser Pro Gly Thr Pro Asn Arg Ile Phe
                2245                2250                2255
Phe Ser Asp Ile His Phe Gly Asn Ile Gln Gln Ile Asn Asp Asp Gly
            2260                2265                2270
Ser Arg Arg Ile Thr Ile Val Glu Asn Val Gly Ser Val Glu Gly Leu
        2275                2280                2285
Ala Tyr His Arg Gly Trp Asp Thr Leu Tyr Trp Thr Ser Tyr Thr Thr
    2290                2295                2300
Ser Thr Ile Thr Arg His Thr Val Asp Gln Thr Arg Pro Gly Ala Phe
2305                2310                2315                2320
Glu Arg Glu Thr Val Ile Thr Met Ser Gly Asp Asp His Pro Arg Ala
                2325                2330                2335
Phe Val Leu Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp Asn
            2340                2345                2350
Glu Gln His Pro Ser Ile Met Arg Ala Ala Leu Ser Gly Ala Asn Val
        2355                2360                2365
Leu Thr Leu Ile Glu Lys Asp Ile Arg Thr Pro Asn Gly Leu Ala Ile
    2370                2375                2380
Asp His Arg Ala Glu Lys Leu Tyr Phe Ser Asp Ala Thr Leu Asp Lys
2385                2390                2395                2400
Ile Glu Arg Cys Glu Tyr Asp Gly Ser His Arg Tyr Val Ile Leu Lys
                2405                2410                2415
Ser Glu Pro Val His Pro Phe Gly Leu Ala Val Tyr Gly Glu His Ile
            2420                2425                2430
Phe Trp Thr Asp Trp Val Arg Arg Ala Val Gln Arg Ala Asn Lys His
        2435                2440                2445
Val Gly Ser Asn Met Lys Leu Leu Arg Val Asp Ile Pro Gln Gln Pro
    2450                2455                2460
Met Gly Ile Ile Ala Val Ala Asn Asp Thr Asn Ser Cys Glu Leu Ser
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2465 2470 2475 2480
Pro Cys Arg Ile Asn Asn Gly Gly Cys Gln Asp Leu Cys Leu Leu Thr
2485 2490 2495
His Gln Gly His Val Asn Cys Ser Cys Arg Gly Gly Arg Ile Leu Gln
2500 2505 2510
Asp Asp Leu Thr Cys Arg Ala Val Asn Ser Ser Cys Arg Ala Gln Asp
2515 2520 2525
Glu Phe Glu Cys Ala Asn Gly Glu Cys Ile Asn Phe Ser Leu Thr Cys
2530 2535 2540
Asp Gly Val Pro His Cys Lys Asp Lys Ser Asp Glu Lys Pro Ser Tyr
2545 2550 2555 2560
Cys Asn Ser Arg Arg Cys Lys Lys Thr Phe Arg Gln Cys Ser Asn Gly
2565 2570 2575
Arg Cys Val Ser Asn Met Leu Trp Cys Asn Gly Ala Asp Asp Cys Gly
2580 2585 2590
Asp Gly Ser Asp Glu Ile Pro Cys Asn Lys Thr Ala Cys Gly Val Gly
2595 2600 2605
Glu Phe Arg Cys Arg Asp Gly Thr Cys Ile Gly Asn Ser Ser Arg Cys
2610 2615 2620
Asn Gln Phe Val Asp Cys Glu Asp Ala Ser Asp Glu Met Asn Cys Ser
2625 2630 2635 2640
Ala Thr Asp Cys Ser Ser Tyr Phe Arg Leu Gly Val Lys Gly Val Leu
2645 2650 2655
Phe Gln Pro Cys Glu Arg Thr Ser Leu Cys Tyr Ala Pro Ser Trp Val
2660 2665 2670
Cys Asp Gly Ala Asn Asp Cys Gly Asp Tyr Ser Asp Glu Arg Asp Cys
2675 2680 2685
Pro Gly Val Lys Arg Pro Arg Cys Pro Leu Asn Tyr Phe Ala Cys Pro
2690 2695 2700
Ser Gly Arg Cys Ile Pro Met Ser Trp Thr Cys Asp Lys Glu Asp Asp
2705 2710 2715 2720
Cys Glu His Gly Glu Asp Glu Thr His Cys Asn Lys Phe Cys Ser Glu
2725 2730 2735
Ala Gln Phe Glu Cys Gln Asn His Arg Cys Ile Ser Lys Gln Trp Leu
2740 2745 2750
Cys Asp Gly Ser Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ala His
2755 2760 2765
Cys Glu Gly Lys Thr Cys Gly Pro Ser Ser Phe Ser Cys Pro Gly Thr
2770 2775 2780
His Val Cys Val Pro Glu Arg Trp Leu Cys Asp Gly Asp Lys Asp Cys
2785 2790 2795 2800
Ala Asp Gly Ala Asp Glu Ser Ile Ala Ala Gly Cys Leu Tyr Asn Ser
2805 2810 2815
Thr Cys Asp Asp Arg Glu Phe Met Cys Gln Asn Arg Gln Cys Ile Pro
2820 2825 2830
Lys His Phe Val Cys Asp His Asp Arg Asp Cys Ala Asp Gly Ser Asp
2835 2840 2845
Glu Ser Pro Glu Cys Glu Tyr Pro Thr Cys Gly Pro Ser Glu Phe Arg
2850 2855 2860
Cys Ala Asn Gly Arg Cys Leu Ser Ser Arg Gln Trp Glu Cys Asp Gly
2865 2870 2875 2880
Glu Asn Asp Cys His Asp Gln Ser Asp Glu Ala Pro Lys Asn Pro His

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            2885                2890                2895
Cys Thr Ser Pro Glu His Lys Cys Asn Ala Ser Ser Gln Phe Leu Cys
        2900                2905                2910
Ser Ser Gly Arg Cys Val Ala Glu Ala Leu Leu Cys Asn Gly Gln Asp
    2915                2920                2925
Asp Cys Gly Asp Ser Ser Asp Glu Arg Gly Cys His Ile Asn Glu Cys
2930                2935                2940
Leu Ser Arg Lys Leu Ser Gly Cys Ser Gln Asp Cys Glu Asp Leu Lys
2945                2950                2955                2960
Ile Gly Phe Lys Cys Arg Cys Arg Pro Gly Phe Arg Leu Lys Asp Asp
            2965                2970                2975
Gly Arg Thr Cys Ala Asp Val Asp Glu Cys Ser Thr Thr Phe Pro Cys
        2980                2985                2990
Ser Gln Arg Cys Ile Asn Thr His Gly Ser Tyr Lys Cys Leu Cys Val
    2995                3000                3005
Glu Gly Tyr Ala Pro Arg Gly Gly Asp Pro His Ser Cys Lys Ala Val
3010                3015                3020
Thr Asp Glu Glu Pro Phe Leu Ile Phe Ala Asn Arg Tyr Tyr Leu Arg
3025                3030                3035                3040
Lys Leu Asn Leu Asp Gly Ser Asn Tyr Thr Leu Leu Lys Gln Gly Leu
            3045                3050                3055
Asn Asn Ala Val Ala Leu Asp Phe Asp Tyr Arg Glu Gln Met Ile Tyr
        3060                3065                3070
Trp Thr Asp Val Thr Thr Gln Gly Ser Met Ile Arg Arg Met His Leu
    3075                3080                3085
Asn Gly Ser Asn Val Gln Val Leu His Arg Thr Gly Leu Ser Asn Pro
3090                3095                3100
Asp Gly Leu Ala Val Asp Trp Val Gly Gly Asn Leu Tyr Trp Cys Asp
3105                3110                3115                3120
Lys Gly Arg Asp Thr Ile Glu Val Ser Lys Leu Asn Gly Ala Tyr Arg
            3125                3130                3135
Thr Val Leu Val Ser Ser Gly Leu Arg Glu Pro Arg Ala Leu Val Val
        3140                3145                3150
Asp Val Gln Asn Gly Tyr Leu Tyr Trp Thr Asp Trp Gly Asp His Ser
    3155                3160                3165
Leu Ile Gly Arg Ile Gly Met Asp Gly Ser Ser Arg Ser Val Ile Val
3170                3175                3180
Asp Thr Lys Ile Thr Trp Pro Asn Gly Leu Thr Leu Asp Tyr Val Thr
3185                3190                3195                3200
Glu Arg Ile Tyr Trp Ala Asp Ala Arg Glu Asp Tyr Ile Glu Phe Ala
            3205                3210                3215
Ser Leu Asp Gly Ser Asn Arg His Val Val Leu Ser Gln Asp Ile Pro
        3220                3225                3230
His Ile Phe Ala Leu Thr Leu Phe Glu Asp Tyr Val Tyr Trp Thr Asp
    3235                3240                3245
Trp Glu Thr Lys Ser Ile Asn Arg Ala His Lys Thr Thr Gly Thr Asn
3250                3255                3260
Lys Thr Leu Leu Ile Ser Thr Leu His Arg Pro Met Asp Leu His Val
3265                3270                3275                3280
Phe His Ala Leu Arg Gln Pro Asp Val Pro Asn His Pro Cys Lys Val
            3285                3290                3295
Asn Asn Gly Gly Cys Ser Asn Leu Cys Leu Leu Ser Pro Gly Gly Gly
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            3300                3305                3310
His Lys Cys Ala Cys Pro Thr Asn Phe Tyr Leu Gly Ser Asp Gly Arg
        3315                3320                3325
Thr Cys Val Ser Asn Cys Thr Ala Ser Gln Phe Val Cys Lys Asn Asp
    3330                3335                3340
Lys Cys Ile Pro Phe Trp Trp Lys Cys Asp Thr Glu Asp Asp Cys Gly
3345                3350                3355                3360
Asp His Ser Asp Glu Pro Pro Asp Cys Pro Glu Phe Lys Cys Arg Pro
                3365                3370                3375
Gly Gln Phe Gln Cys Ser Thr Gly Ile Cys Thr Asn Pro Ala Phe Ile
            3380                3385                3390
Cys Asp Gly Asp Asn Asp Cys Gln Asp Asn Ser Asp Glu Ala Asn Cys
        3395                3400                3405
Asp Ile His Val Cys Leu Pro Ser Gln Phe Lys Cys Thr Asn Thr Asn
    3410                3415                3420
Arg Cys Ile Pro Gly Ile Phe Arg Cys Asn Gly Gln Asp Asn Cys Gly
3425                3430                3435                3440
Asp Gly Glu Asp Glu Arg Asp Cys Pro Glu Val Thr Cys Ala Pro Asn
                3445                3450                3455
Gln Phe Gln Cys Ser Ile Thr Lys Arg Cys Ile Pro Arg Val Trp Val
            3460                3465                3470
Cys Asp Arg Asp Asn Asp Cys Val Asp Gly Ser Asp Glu Pro Ala Asn
        3475                3480                3485
Cys Thr Gln Met Thr Cys Gly Val Asp Glu Phe Arg Cys Lys Asp Ser
    3490                3495                3500
Gly Arg Cys Ile Pro Ala Arg Trp Lys Cys Asp Gly Glu Asp Asp Cys
3505                3510                3515                3520
Gly Asp Gly Ser Asp Glu Pro Lys Glu Glu Cys Asp Glu Arg Thr Cys
                3525                3530                3535
Glu Pro Tyr Gln Phe Arg Cys Lys Asn Asn Arg Cys Val Pro Gly Arg
            3540                3545                3550
Trp Gln Cys Asp Tyr Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Glu
        3555                3560                3565
Ser Cys Thr Pro Arg Pro Cys Ser Glu Ser Glu Phe Ser Cys Ala Asn
    3570                3575                3580
Gly Arg Cys Ile Ala Gly Arg Trp Lys Cys Asp Gly Asp His Asp Cys
3585                3590                3595                3600
Ala Asp Gly Ser Asp Glu Lys Asp Cys Thr Pro Arg Cys Asp Met Asp
                3605                3610                3615
Gln Phe Gln Cys Lys Ser Gly His Cys Ile Pro Leu Arg Trp Arg Cys
            3620                3625                3630
Asp Ala Asp Ala Asp Cys Met Asp Gly Ser Asp Glu Glu Ala Cys Gly
        3635                3640                3645
Thr Gly Val Arg Thr Cys Pro Leu Asp Glu Phe Gln Cys Asn Asn Thr
    3650                3655                3660
Leu Cys Lys Pro Leu Ala Trp Lys Cys Asp Gly Glu Asp Asp Cys Gly
3665                3670                3675                3680
Asp Asn Ser Asp Glu Asn Pro Glu Glu Cys Ala Arg Phe Val Cys Pro
                3685                3690                3695
Pro Asn Arg Pro Phe Arg Cys Lys Asn Asp Arg Val Cys Leu Trp Ile
            3700                3705                3710
Gly Arg Gln Cys Asp Gly Thr Asp Asn Cys Gly Asp Gly Thr Asp Glu
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3715 3720 3725
Glu Asp Cys Glu Pro Pro Thr Ala His Thr Thr His Cys Lys Asp Lys
3730 3735 3740
Lys Glu Phe Leu Cys Arg Asn Gln Arg Cys Leu Ser Ser Ser Leu Arg
3745 3750 3755 3760
Cys Asn Met Phe Asp Asp Cys Gly Asp Gly Ser Asp Glu Glu Asp Cys
3765 3770 3775
Ser Ile Asp Pro Lys Leu Thr Ser Cys Ala Thr Asn Ala Ser Ile Cys
3780 3785 3790
Gly Asp Glu Ala Arg Cys Val Arg Thr Glu Lys Ala Ala Tyr Cys Ala
3795 3800 3805
Cys Arg Ser Gly Phe His Thr Val Pro Gly Gln Pro Gly Cys Gln Asp
3810 3815 3820
Ile Asn Glu Cys Leu Arg Phe Gly Thr Cys Ser Gln Leu Cys Asn Asn
3825 3830 3835 3840
Thr Lys Gly Gly His Leu Cys Ser Cys Ala Arg Asn Phe Met Lys Thr
3845 3850 3855
His Asn Thr Cys Lys Ala Glu Gly Ser Glu Tyr Gln Val Leu Tyr Ile
3860 3865 3870
Ala Asp Asp Asn Glu Ile Arg Ser Leu Phe Pro Gly His Pro His Ser
3875 3880 3885
Ala Tyr Glu Gln Ala Phe Gln Gly Asp Glu Ser Val Arg Ile Asp Ala
3890 3895 3900
Met Asp Val His Val Lys Ala Gly Arg Val Tyr Trp Thr Asn Trp His
3905 3910 3915 3920
Thr Gly Thr Ile Ser Tyr Arg Ser Leu Pro Pro Ala Ala Pro Pro Thr
3925 3930 3935
Thr Ser Asn Arg His Arg Arg Gln Ile Asp Arg Gly Val Thr His Leu
3940 3945 3950
Asn Ile Ser Gly Leu Lys Met Pro Arg Gly Ile Ala Ile Asp Trp Val
3955 3960 3965
Ala Gly Asn Val Tyr Trp Thr Asp Ser Gly Arg Asp Val Ile Glu Val
3970 3975 3980
Ala Gln Met Lys Gly Glu Asn Arg Lys Thr Leu Ile Ser Gly Met Ile
3985 3990 3995 4000
Asp Glu Pro His Ala Ile Val Val Asp Pro Leu Arg Gly Thr Met Tyr
4005 4010 4015
Trp Ser Asp Trp Gly Asn His Pro Lys Ile Glu Thr Ala Ala Met Asp
4020 4025 4030
Gly Thr Leu Arg Glu Thr Leu Val Gln Asp Asn Ile Gln Trp Pro Thr
4035 4040 4045
Gly Leu Ala Val Asp Tyr His Asn Glu Arg Leu Tyr Trp Ala Asp Ala
4050 4055 4060
Lys Leu Ser Val Ile Gly Ser Ile Arg Leu Asn Gly Thr Asp Pro Ile
4065 4070 4075 4080
Val Ala Ala Asp Ser Lys Arg Gly Leu Ser His Pro Phe Ser Ile Asp
4085 4090 4095
Val Phe Glu Asp Tyr Ile Tyr Gly Val Thr Tyr Ile Asn Asn Arg Val
4100 4105 4110
Phe Lys Ile His Lys Phe Gly His Ser Pro Leu Val Asn Leu Thr Gly
4115 4120 4125
Gly Leu Ser His Ala Ser Asp Val Val Leu Tyr His Gln His Lys Gln

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    4130                4135                4140
Pro Glu Val Thr Asn Pro Cys Asp Arg Lys Lys Cys Glu Trp Leu Cys
4145                4150                4155                4160
Leu Leu Ser Pro Ser Gly Pro Val Cys Thr Cys Pro Asn Gly Lys Arg
                4165                4170                4175
Leu Asp Asn Gly Thr Cys Val Pro Val Pro Ser Pro Thr Pro Pro Pro
            4180                4185                4190
Asp Ala Pro Arg Pro Gly Thr Cys Asn Leu Gln Cys Phe Asn Gly Gly
        4195                4200                4205
Ser Cys Phe Leu Asn Ala Arg Arg Gln Pro Lys Cys Arg Cys Gln Pro
    4210                4215                4220
Arg Tyr Thr Gly Asp Lys Cys Glu Leu Asp Gln Cys Trp Glu His Cys
4225                4230                4235                4240
Arg Asn Gly Gly Thr Cys Ala Ala Ser Pro Ser Gly Met Pro Thr Cys
                4245                4250                4255
Arg Cys Pro Thr Gly Phe Thr Gly Pro Lys Cys Thr Gln Gln Val Cys
            4260                4265                4270
Ala Gly Tyr Cys Ala Asn Asn Ser Thr Cys Thr Val Asn Gln Gly Asn
        4275                4280                4285
Gln Pro Gln Cys Arg Cys Leu Pro Gly Phe Leu Gly Asp Arg Cys Gln
    4290                4295                4300
Tyr Arg Gln Cys Ser Gly Tyr Cys Glu Asn Phe Gly Thr Cys Gln Met
4305                4310                4315                4320
Ala Ala Asp Gly Ser Arg Gln Cys Arg Cys Thr Ala Tyr Phe Glu Gly
                4325                4330                4335
Ser Arg Cys Glu Val Asn Lys Cys Ser Arg Cys Leu Glu Gly Ala Cys
            4340                4345                4350
Val Val Asn Lys Gln Ser Gly Asp Val Thr Cys Asn Cys Thr Asp Gly
        4355                4360                4365
Arg Val Ala Pro Ser Cys Leu Thr Cys Val Gly His Cys Ser Asn Gly
    4370                4375                4380
Gly Ser Cys Thr Met Asn Ser Lys Met Met Pro Glu Cys Gln Cys Pro
4385                4390                4395                4400
Pro His Met Thr Gly Pro Arg Cys Glu Glu His Val Phe Ser Gln Gln
                4405                4410                4415
Gln Pro Gly His Ile Ala Ser Ile Leu Ile Pro Leu Leu Leu Leu Leu
            4420                4425                4430
Leu Leu Val Leu Val Ala Gly Val Val Phe Trp Tyr Lys Arg Arg Val
        4435                4440                4445
Gln Gly Ala Lys Gly Phe Gln His Gln Arg Met Thr Asn Gly Ala Met
    4450                4455                4460
Asn Val Glu Ile Gly Asn Pro Thr Tyr Lys Met Tyr Glu Gly Gly Glu
4465                4470                4475                4480
Pro Asp Asp Val Gly Gly Leu Leu Asp Ala Asp Phe Ala Leu Asp Pro
                4485                4490                4495
Asp Lys Pro Thr Asn Phe Thr Asn Pro Val Tyr Ala Thr Leu Tyr Met
            4500                4505                4510
Gly Gly His Gly Ser Arg His Ser Leu Ala Ser Thr Asp Glu Lys Arg
        4515                4520                4525
Glu Leu Leu Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu Ala
    4530                4535                4540
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<211> 478
<212> DNA
<213> Mus musculus

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<221> misc_feature
<222> 15
<223> n is any

<400> 11
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ctttcctgca gggccagcca aagtatcagc aacaacctac actggtatca acaaaaatca
120
catgagtctc caaggattct catcaagtat gcatcccagt ccatctatgg gatcccctca
180
aggttcagtg gcagtggatc agggacattt ttcactctca ttgtcaacag tgtggggact
240
gaagattttg gaatgtattt ctgtcaacag agtcacagct ggcctctcac gttcggtact
300
gggaccaagc tggagctgaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
360
tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
420
cccagagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcc
478

<210> 12
<211> 159
<212> PRT
<213> Mus musculus

<400> 12
Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Asp Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
20 25 30
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Ile Leu Ile
35 40 45
Lys Tyr Ala Ser Gln Ser Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Phe Phe Thr Leu Ile Val Asn Ser Val Gly Thr
65 70 75 80
Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser His Ser Trp Pro Leu
85 90 95
Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala
100 105 110
Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115 120 125
Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Arg Asp Ile
130 135 140

Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val
145 150 155

<210> 13
<211> 366
<212> DNA
<213> Mus musculus

<400> 13
gaggttaagy tkgttgartc tggaggagac ttagtgaaac ctggagggtc cctgaaactc
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gcctgtgcag cctctggatt cactttcagt aacgatgcca tgtcttgggt tcgccagact
120
ccagaaaaga ggctggagtg ggtcgcatcc attagtagtg ttggtaacac ctactatcca
180
gacagtgtga agggccgatt caccatctcc agagataatg ccaggaacat tctatacctg
240
caaatgagta gtgtgaggtc tgaggacacg gccatgtatt actgtgcaag aggctatggt
300
gttagtccct ggttttctta ctggggccaa gggactctag tcaccgtctc ctcagccaaa
360
acaaca
366

<210> 14
<211> 122
<212> PRT
<213> Mus musculus

<400> 14
Glu Val Lys Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ala Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Asp
20 25 30
Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45
Ala Ser Ile Ser Ser Val Gly Asn Thr Tyr Tyr Pro Asp Ser Val Lys
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
65 70 75 80
Gln Met Ser Ser Val Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95
Arg Gly Tyr Gly Val Ser Pro Trp Phe Ser Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Lys Thr Thr
115 120

<210> 15
<211> 493
<212> DNA
<213> Mus musculus

<400> 15
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60
atctcttgca gatctagtca gaacattgta catagtagtg gaaacaccta tttagaatgg
120
tacctgcaga aaccaggcca gtctccaaag ctcctgatct acaaagtttc caaccgattt
180
tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc
240
agcagagtgg aggctgagga tctgggaatt tattactgct ttcaaggttc acatgttccg
300
tacacgttcg gaggggggac caagctggaa ataaaacggg ctgatgctgc accaactgta
360
tccatcttcc caccatccag tgagcagtta acatctggag gtgcctcagt cgtgtgcttc
420
ttgaacaact tctaccccag agacatcaat gtcaagtgga agattgatgg cagtgaacga
480
caaaatggcg tcc
493

<210> 16
<211> 164
<212> PRT
<213> Mus musculus

<400> 16
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
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Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser
            20   25   30
Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35   40   45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50   55   60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65   70   75   80
Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly
                85   90   95
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100   105   110
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
        115   120   125
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
    130   135   140
Tyr Pro Arg Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
145   150   155   160
Gln Asn Gly Val

<210> 17
<211> 354
<212> DNA

<213> Mus musculus

<400> 17
gaggtyatgy tkgtygartc tggaggaggc ttagtgaagc ctggagggtc cctgaaactc
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tcctgtgtag cctctggatt cactttcagt agatatacca tgtcttgggt tcgccagact
120
ccggcgaaga gactggagtg ggtcgcaacc atcaattttg gtaatggtaa cacctactat
180
cctgacagtg tgaagggccg attcaccatc tccagagaca atgccaggaa caccctgtat
240
ctgcaaatga gcagtctgag gtctgaggac acggccatgt attactgtac aagccttaat
300
tgggcttact ggggccaagg gactctggtc accgtctcct cagccaaaac aaca
354

<210> 18
<211> 118
<212> PRT
<213> Mus musculus

<400> 18
Glu Val Met Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30
Thr Met Ser Trp Val Arg Gln Thr Pro Ala Lys Arg Leu Glu Trp Val
35 40 45
Ala Thr Ile Asn Phe Gly Asn Gly Asn Thr Tyr Tyr Pro Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Thr Ser Leu Asn Trp Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
100 105 110
Ser Ser Ala Lys Thr Thr
115

<210> 19
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide fragment representing
Amyloid-beta 35-42 region

<400> 19
Cys Met Val Gly Gly Val Val Ile Ala
1 5

<210> 20
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence representing
Amyloid-beta 1-12 region

<400> 20
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val Cys
1 5 10

<210> 21
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence representing
carboxyl-terminal 13 amino acid peptide of LRP
with amino-terminal cysteine

<400> 21
Cys Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu Ala
1 5 10

<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 22
ggacgccatt ttgtcgttca ctgcca
26

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 23
tgttgttttg gctgaggaga cggtga
26

<210> 24

<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<221> misc_feature
<222> 15, 24
<223> N is any

<400> 24
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26

<210> 25
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<212> DNA
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<220>
<223> Primer

<400> 25
gargtyaagy tbgtygartc yggagg
26

<210> 26
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 26
gaygtyytba tgacycarac ycca
24

<210> 27
<211> 2256
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(2256)
<223> APP 751

<300>
<308> Genbank X06989
<309> 2001-11-15

<400> 27

atg ctg ccc ggt ttg gca ctg ctc ctg ctg gcc gcc tgg acg gct cgg 48
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
1 5 10 15

gcg ctg gag gta ccc act gat ggt aat gct ggc ctg ctg gct gaa ccc 96
Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
20 25 30

cag att gcc atg ttc tgt ggc aga ctg aac atg cac atg aat gtc cag 144
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

aat ggg aag tgg gat tca gat cca tca ggg acc aaa acc tgc att gat 192
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

acc aag gaa ggc atc ctg cag tat tgc caa gaa gtc tac cct gaa ctg 240
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80

cag atc acc aat gtg gta gaa gcc aac caa cca gtg acc atc cag aac 288
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

tgg tgc aag cgg ggc cgc aag cag tgc aag acc cat ccc cac ttt gtg 336
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110

att ccc tac cgc tgc tta gtt ggt gag ttt gta agt gat gcc ctt ctc 384
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125

gtt cct gac aag tgc aaa ttc tta cac cag gag agg atg gat gtt tgc 432
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130 135 140

gaa act cat ctt cac tgg cac acc gtc gcc aaa gag aca tgc agt gag 480
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

aag agt acc aac ttg cat gac tac ggc atg ttg ctg ccc tgc gga att 528
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
165 170 175

gac aag ttc cga ggg gta gag ttt gtg tgt tgc cca ctg gct gaa gaa 576
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
180 185 190

agt gac aat gtg gat tct gct gat gcg gag gag gat gac tcg gat gtc 624
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val

195 200 205

tgg tgg ggc gga gca gac aca gac tat gca gat ggg agt gaa gac aaa 672
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210 215 220

gta gta gaa gta gca gag gag gaa gaa gtg gct gag gtg gaa gaa gaa 720
Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

gaa gcc gat gat gac gag gac gat gag gat ggt gat gag gta gag gaa 768
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

gag gct gag gaa ccc tac gaa gaa gcc aca gag aga acc acc agc att 816
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

gcc acc acc acc acc acc acc aca gag tct gtg gaa gag gtg gtt cga 864
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

gag gtg tgc tct gaa caa gcc gag acg ggg ccg tgc cga gca atg atc 912
Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
290 295 300

tcc cgc tgg tac ttt gat gtg act gaa ggg aag tgt gcc cca ttc ttt 960
Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305 310 315 320

tac ggc gga tgt ggc ggc aac cgg aac aac ttt gac aca gaa gag tac 1008
Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu Tyr
325 330 335

tgc atg gcc gtg tgt ggc agc gcc att cct aca aca gca gcc agt acc 1056
Cys Met Ala Val Cys Gly Ser Ala Ile Pro Thr Thr Ala Ala Ser Thr
340 345 350

cct gat gcc gtt gac aag tat ctc gag aca cct ggg gat gag aat gaa 1104
Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp Glu Asn Glu
355 360 365

cat gcc cat ttc cag aaa gcc aaa gag agg ctt gag gcc aag cac cga 1152
His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala Lys His Arg
370 375 380

gag aga atg tcc cag gtc atg aga gaa tgg gaa gag gca gaa cgt caa 1200
Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala Glu Arg Gln
385 390 395 400

gca aag aac ttg cct aaa gct gat aag aag gca gtt atc cag cat ttc 1248
Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile Gln His Phe

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                405                 410                 415

cag gag aaa gtg gaa tct ttg gaa cag gaa gca gcc aac gag aga cag    1296
Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn Glu Arg Gln
            420                 425                 430

cag ctg gtg gag aca cac atg gcc aga gtg gaa gcc atg ctc aat gac    1344
Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met Leu Asn Asp
        435                 440                 445

cgc cgc cgc ctg gcc ctg gag aac tac atc acc gct ctg cag gct gtt    1392
Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu Gln Ala Val
    450                 455                 460

cct cct cgg cct cgt cac gtg ttc aat atg cta aag aag tat gtc cgc    1440
Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys Tyr Val Arg
465                 470                 475                 480

gca gaa cag aag gac aga cag cac acc cta aag cat ttc gag cat gtg    1488
Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe Glu His Val
                485                 490                 495

cgc atg gtg gat ccc aag aaa gcc gct cag atc cgg tcc cag gtt atg    1536
Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser Gln Val Met
            500                 505                 510

aca cac ctc cgt gtg att tat gag cgc atg aat cag tct ctc tcc ctg    1584
Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu
        515                 520                 525

ctc tac aac gtg cct gca gtg gcc gag gag att cag gat gaa gtt gat    1632
Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp Glu Val Asp
    530                 535                 540

gag ctg ctt cag aaa gag caa aac tat tca gat gac gtc ttg gcc aac    1680
Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn
545                 550                 555                 560

atg att agt gaa cca agg atc agt tac gga aac gat gct ctc atg cca    1728
Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro
                565                 570                 575

tct ttg acc gaa acg aaa acc acc gtg gag ctc ctt ccc gtg aat gga    1776
Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro Val Asn Gly
            580                 585                 590

gag ttc agc ctg gac gat ctc cag ccg tgg cat tct ttt ggg gct gac    1824
Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp
        595                 600                 605

tct gtg cca gcc aac aca gaa aac gaa gtt gag cct gtt gat gcc cgc    1872
Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg
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    610                 615                 620

cct gct gcc gac cga gga ctg acc act cga cca ggt tct ggg ttg aca     1920
Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr
625                 630                 635                 640

aat atc aag acg gag gag atc tct gaa gtg aag atg gat gca gaa ttc     1968
Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe
                645                 650                 655

cga cat gac tca gga tat gaa gtt cat cat caa aaa ttg gtg ttc ttt     2016
Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe
            660                 665                 670

gca gaa gat gtg ggt tca aac aaa ggt gca atc att gga ctc atg gtg     2064
Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val
        675                 680                 685

ggc ggt gtt gtc ata gcg aca gtg atc gtc atc acc ttg gtg atg ctg     2112
Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu
    690                 695                 700

aag aag aaa cag tac aca tcc att cat cat ggt gtg gtg gag gtt gac     2160
Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp
705                 710                 715                 720

gcc gct gtc acc cca gag gag cgc cac ctg tcc aag atg cag cag aac     2208
Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn
                725                 730                 735

ggc tac gaa aat cca acc tac aag ttc ttt gag cag atg cag aac tag     2256
Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn
            740                 745                 750


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Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45
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Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220
Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285
Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
    290                 295                 300
Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305                 310                 315                 320
Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu Tyr
                325                 330                 335
Cys Met Ala Val Cys Gly Ser Ala Ile Pro Thr Thr Ala Ala Ser Thr
            340                 345                 350
Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp Glu Asn Glu
        355                 360                 365
His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala Lys His Arg
    370                 375                 380
Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala Glu Arg Gln
385                 390                 395                 400
Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile Gln His Phe
                405                 410                 415
Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn Glu Arg Gln
            420                 425                 430
Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met Leu Asn Asp
        435                 440                 445
Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu Gln Ala Val
    450                 455                 460
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Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys Tyr Val Arg
465                 470                 475                 480
Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe Glu His Val
                485                 490                 495
Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser Gln Val Met
            500                 505                 510
Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu
        515                 520                 525
Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp Glu Val Asp
    530                 535                 540
Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn
545                 550                 555                 560
Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro
                565                 570                 575
Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro Val Asn Gly
            580                 585                 590
Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp
        595                 600                 605
Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg
    610                 615                 620
Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr
625                 630                 635                 640
Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe
                645                 650                 655
Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe
            660                 665                 670
Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val
        675                 680                 685
Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu
    690                 695                 700
Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp
705                 710                 715                 720
Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn
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1               5                   10                  15

gcg ctg gag gta ccc act gat ggt aat gct ggc ctg ctg gct gaa ccc      96
Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

cag att gcc atg ttc tgt ggc aga ctg aac atg cac atg aat gtc cag     144
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

aat ggg aag tgg gat tca gat cca tca ggg acc aaa acc tgc att gat     192
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

acc aag gaa ggc atc ctg cag tat tgc caa gaa gtc tac cct gaa ctg     240
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

cag atc acc aat gtg gta gaa gcc aac caa cca gtg acc atc cag aac     288
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

tgg tgc aag cgg ggc cgc aag cag tgc aag acc cat ccc cac ttt gtg     336
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

att ccc tac cgc tgc tta gtt ggt gag ttt gta agt gat gcc ctt ctc     384
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

gtt cct gac aag tgc aaa ttc tta cac cag gag agg atg gat gtt tgc     432
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

gaa act cat ctt cac tgg cac acc gtc gcc aaa gag aca tgc agt gag     480
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

aag agt acc aac ttg cat gac tac ggc atg ttg ctg ccc tgc gga att     528
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

gac aag ttc cga ggg gta gag ttt gtg tgt tgc cca ctg gct gaa gaa     576
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

agt gac aat gtg gat tct gct gat gcg gag gag gat gac tcg gat gtc     624
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205
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tgg tgg ggc gga gca gac aca gac tat gca gat ggg agt gaa gac aaa 672
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210 215 220

gta gta gaa gta gca gag gag gaa gaa gtg gct gag gtg gaa gaa gaa 720
Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

gaa gcc gat gat gac gag gac gat gag gat ggt gat gag gta gag gaa 768
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

gag gct gag gaa ccc tac gaa gaa gcc aca gag aga acc acc agc att 816
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

gcc acc acc acc acc acc acc aca gag tct gtg gaa gag gtg gtt cga 864
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

gtt cct aca aca gca gcc agt acc cct gat gcc gtt gac aag tat ctc 912
Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290 295 300

gag aca cct ggg gat gag aat gaa cat gcc cat ttc cag aaa gcc aaa 960
Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

gag agg ctt gag gcc aag cac cga gag aga atg tcc cag gtc atg aga 1008
Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

gaa tgg gaa gag gca gaa cgt caa gca aag aac ttg cct aaa gct gat 1056
Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

aag aag gca gtt atc cag cat ttc cag gag aaa gtg gaa tct ttg gaa 1104
Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

cag gaa gca gcc aac gag aga cag cag ctg gtg gag aca cac atg gcc 1152
Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

aga gtg gaa gcc atg ctc aat gac cgc cgc cgc ctg gcc ctg gag aac 1200
Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

tac atc acc gct ctg cag gct gtt cct cct cgg cct cgt cac gtg ttc 1248
Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

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aat atg cta aag aag tat gtc cgc gca gaa cag aag gac aga cag cac      1296
Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430

acc cta aag cat ttc gag cat gtg cgc atg gtg gat ccc aag aaa gcc      1344
Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
        435                 440                 445

gct cag atc cgg tcc cag gtt atg aca cac ctc cgt gtg att tat gag      1392
Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450                 455                 460

cgc atg aat cag tct ctc tcc ctg ctc tac aac gtg cct gca gtg gcc      1440
Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465                 470                 475                 480

gag gag att cag gat gaa gtt gat gag ctg ctt cag aaa gag caa aac      1488
Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
                485                 490                 495

tat tca gat gac gtc ttg gcc aac atg att agt gaa cca agg atc agt      1536
Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
            500                 505                 510

tac gga aac gat gct ctc atg cca tct ttg acc gaa acg aaa acc acc      1584
Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525

gtg gag ctc ctt ccc gtg aat gga gag ttc agc ctg gac gat ctc cag      1632
Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540

ccg tgg cat tct ttt ggg gct gac tct gtg cca gcc aac aca gaa aac      1680
Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560

gaa gtt gag cct gtt gat gcc cgc cct gct gcc gac cga gga ctg acc      1728
Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575

act cga cca ggt tct ggg ttg aca aat atc aag acg gag gag atc tct      1776
Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590

gaa gtg aag atg gat gca gaa ttc cga cat gac tca gga tat gaa gtt      1824
Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605

cat cat caa aaa ttg gtg ttc ttt gca gaa gat gtg ggt tca aac aaa      1872
His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620
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ggt gca atc att gga ctc atg gtg ggc ggt gtt gtc ata gcg aca gtg 1920
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

atc gtc atc acc ttg gtg atg ctg aag aag aaa cag tac aca tcc att 1968
Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

cat cat ggt gtg gtg gag gtt gac gcc gct gtc acc cca gag gag cgc 2016
His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

cac ctg tcc aag atg cag cag aac ggc tac gaa aat cca acc tac aag 2064
His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
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35 40 45
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65 70 75 80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130 135 140
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

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Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220
Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285
Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
    290                 295                 300
Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305                 310                 315                 320
Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
                325                 330                 335
Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
            340                 345                 350
Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
        355                 360                 365
Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
    370                 375                 380
Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385                 390                 395                 400
Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
                405                 410                 415
Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430
Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
        435                 440                 445
Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450                 455                 460
Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465                 470                 475                 480
Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
                485                 490                 495
Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
            500                 505                 510
Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525
Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540
Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560
Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575
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Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590
Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605
His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625                 630                 635                 640
Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
                645                 650                 655
His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
            660                 665                 670
His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
        675                 680                 685
Phe Phe Glu Gln Met Gln Asn
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Met Pro Pro Leu Leu Ala Pro Leu Leu Cys Leu Ala Leu Leu Pro Ala
 1               5                  10                  15

ctc gcc gca cga ggc ccg cga tgc tcc cag ccc ggt gag acc tgc ctg      96
Leu Ala Ala Arg Gly Pro Arg Cys Ser Gln Pro Gly Glu Thr Cys Leu
            20                  25                  30

aat ggc ggg aag tgt gaa gcg gcc aat ggc acg gag gcc tgc gtc tgt     144
Asn Gly Gly Lys Cys Glu Ala Ala Asn Gly Thr Glu Ala Cys Val Cys
        35                  40                  45

ggc ggg gcc ttc gtg ggc ccg cga tgc cag gac ccc aac ccg tgc ctc     192
Gly Gly Ala Phe Val Gly Pro Arg Cys Gln Asp Pro Asn Pro Cys Leu
    50                  55                  60

agc acc ccc tgc aag aac gcc ggg aca tgc cac gtg gtg gac cgc aga     240
Ser Thr Pro Cys Lys Asn Ala Gly Thr Cys His Val Val Asp Arg Arg
65                  70                  75                  80
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ggc gtg gca gac tat gcc tgc agc tgt gcc ctg ggc ttc tct ggg ccc     288
Gly Val Ala Asp Tyr Ala Cys Ser Cys Ala Leu Gly Phe Ser Gly Pro
                85                  90                  95

ctc tgc ctg aca ccc ctg gac aac gcc tgc ctc acc aac ccc tgc cgc     336
Leu Cys Leu Thr Pro Leu Asp Asn Ala Cys Leu Thr Asn Pro Cys Arg
            100                 105                 110

aac ggg ggc acc tgc gac ctg ctc acg ctg acg gag tac aag tgc cgc     384
Asn Gly Gly Thr Cys Asp Leu Leu Thr Leu Thr Glu Tyr Lys Cys Arg
        115                 120                 125

tgc ccg ccc ggc tgg tca ggg aaa tcg tgc cag cag gct gac ccg tgc     432
Cys Pro Pro Gly Trp Ser Gly Lys Ser Cys Gln Gln Ala Asp Pro Cys
    130                 135                 140

gcc tcc aac ccc tgc gcc aac ggt ggc cag tgc ctg ccc ttc gag gcc     480
Ala Ser Asn Pro Cys Ala Asn Gly Gly Gln Cys Leu Pro Phe Glu Ala
145                 150                 155                 160

tcc tac atc tgc cac tgc cca ccc agc ttc cat ggc ccc acc tgc cgg     528
Ser Tyr Ile Cys His Cys Pro Pro Ser Phe His Gly Pro Thr Cys Arg
                165                 170                 175

cag gat gtc aac gag tgt ggc cag aag ccc agg ctt tgc cgc cac gga     576
Gln Asp Val Asn Glu Cys Gly Gln Lys Pro Arg Leu Cys Arg His Gly
            180                 185                 190

ggc acc tgc cac aac gag gtc ggc tcc tac cgc tgc gtc tgc cgc gcc     624
Gly Thr Cys His Asn Glu Val Gly Ser Tyr Arg Cys Val Cys Arg Ala
        195                 200                 205

acc cac act ggc ccc aac tgc gag cgg ccc tac gtg ccc tgc agc ccc     672
Thr His Thr Gly Pro Asn Cys Glu Arg Pro Tyr Val Pro Cys Ser Pro
    210                 215                 220

tcg ccc tgc cag aac ggg ggc acc tgc cgc ccc acg ggc gac gtc acc     720
Ser Pro Cys Gln Asn Gly Gly Thr Cys Arg Pro Thr Gly Asp Val Thr
225                 230                 235                 240

cac gag tgt gcc tgc ctg cca ggc ttc acc ggc cag aac tgt gag gaa     768
His Glu Cys Ala Cys Leu Pro Gly Phe Thr Gly Gln Asn Cys Glu Glu
                245                 250                 255

aat atc gac gat tgt cca gga aac aac tgc aag aac ggg ggt gcc tgt     816
Asn Ile Asp Asp Cys Pro Gly Asn Asn Cys Lys Asn Gly Gly Ala Cys
            260                 265                 270

gtg gac ggc gtg aac acc tac aac tgc ccg tgc ccg cca gag tgg aca     864
Val Asp Gly Val Asn Thr Tyr Asn Cys Pro Cys Pro Pro Glu Trp Thr
        275                 280                 285
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ggt cag tac tgt acc gag gat gtg gac gag tgc cag ctg atg cca aat     912
Gly Gln Tyr Cys Thr Glu Asp Val Asp Glu Cys Gln Leu Met Pro Asn
    290                 295                 300

gcc tgc cag aac ggc ggg acc tgc cac aac acc cac ggt ggc tac aac     960
Ala Cys Gln Asn Gly Gly Thr Cys His Asn Thr His Gly Gly Tyr Asn
305                 310                 315                 320

tgc gtg tgt gtc aac ggc tgg act ggt gag gac tgc agc gag aac att    1008
Cys Val Cys Val Asn Gly Trp Thr Gly Glu Asp Cys Ser Glu Asn Ile
                325                 330                 335

gat gac tgt gcc agc gcc gcc tgc ttc cac ggc gcc acc tgc cat gac    1056
Asp Asp Cys Ala Ser Ala Ala Cys Phe His Gly Ala Thr Cys His Asp
            340                 345                 350

cgt gtg gcc tcc ttt tac tgc gag tgt ccc cat ggc cgc aca ggt ctg    1104
Arg Val Ala Ser Phe Tyr Cys Glu Cys Pro His Gly Arg Thr Gly Leu
        355                 360                 365

ctg tgc cac ctc aac gac gca tgc atc agc aac ccc tgt aac gag ggc    1152
Leu Cys His Leu Asn Asp Ala Cys Ile Ser Asn Pro Cys Asn Glu Gly
    370                 375                 380

tcc aac tgc gac acc aac cct gtc aat ggc aag gcc atc tgc acc tgc    1200
Ser Asn Cys Asp Thr Asn Pro Val Asn Gly Lys Ala Ile Cys Thr Cys
385                 390                 395                 400

ccc tcg ggg tac acg ggc ccg gcc tgc agc cag gac gtg gat gag tgc    1248
Pro Ser Gly Tyr Thr Gly Pro Ala Cys Ser Gln Asp Val Asp Glu Cys
                405                 410                 415

tcg ctg ggt gcc aac ccc tgc gag cat gcg ggc aag tgc atc aac acg    1296
Ser Leu Gly Ala Asn Pro Cys Glu His Ala Gly Lys Cys Ile Asn Thr
            420                 425                 430

ctg ggc tcc ttc gag tgc cag tgt ctg cag ggc tac acg ggc ccc cga    1344
Leu Gly Ser Phe Glu Cys Gln Cys Leu Gln Gly Tyr Thr Gly Pro Arg
        435                 440                 445

tgc gag atc gac gtc aac gag tgc gtc tcg aac ccg tgc cag aac gac    1392
Cys Glu Ile Asp Val Asn Glu Cys Val Ser Asn Pro Cys Gln Asn Asp
    450                 455                 460

gcc acc tgc ctg gac cag att ggg gag ttc cag tgc atg tgc atg ccc    1440
Ala Thr Cys Leu Asp Gln Ile Gly Glu Phe Gln Cys Met Cys Met Pro
465                 470                 475                 480

ggc tac gag ggt gtg cac tgc gag gtc aac aca gac gag tgt gcc agc    1488
Gly Tyr Glu Gly Val His Cys Glu Val Asn Thr Asp Glu Cys Ala Ser
                485                 490                 495
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agc ccc tgc ctg cac aat ggc cgc tgc ctg gac aag atc aat gag ttc    1536
Ser Pro Cys Leu His Asn Gly Arg Cys Leu Asp Lys Ile Asn Glu Phe
            500                 505                 510

cag tgc gag tgc ccc acg ggc ttc act ggg cat ctg tgc cag tac gat    1584
Gln Cys Glu Cys Pro Thr Gly Phe Thr Gly His Leu Cys Gln Tyr Asp
        515                 520                 525

gtg gac gag tgt gcc agc acc ccc tgc aag aat ggt gcc aag tgc ctg    1632
Val Asp Glu Cys Ala Ser Thr Pro Cys Lys Asn Gly Ala Lys Cys Leu
    530                 535                 540

gac gga ccc aac act tac acc tgt gtg tgc acg gaa ggg tac acg ggg    1680
Asp Gly Pro Asn Thr Tyr Thr Cys Val Cys Thr Glu Gly Tyr Thr Gly
545                 550                 555                 560

acg cac tgc gag gtg gac atc gat gag tgc gac ccc gac ccc tgc cac    1728
Thr His Cys Glu Val Asp Ile Asp Glu Cys Asp Pro Asp Pro Cys His
                565                 570                 575

tac ggc tcc tgc aag gac ggc gtc gcc acc ttc acc tgc ctc tgc cgc    1776
Tyr Gly Ser Cys Lys Asp Gly Val Ala Thr Phe Thr Cys Leu Cys Arg
            580                 585                 590

cca ggc tac acg ggc cac cac tgc gag acc aac atc aac gag tgc tcc    1824
Pro Gly Tyr Thr Gly His His Cys Glu Thr Asn Ile Asn Glu Cys Ser
        595                 600                 605

agc cag ccc tgc cgc cta cgg ggc acc tgc cag gac ccg gac aac gcc    1872
Ser Gln Pro Cys Arg Leu Arg Gly Thr Cys Gln Asp Pro Asp Asn Ala
    610                 615                 620

tac ctc tgc ttc tgc ctg aag ggg acc aca gga ccc aac tgc gag atc    1920
Tyr Leu Cys Phe Cys Leu Lys Gly Thr Thr Gly Pro Asn Cys Glu Ile
625                 630                 635                 640

aac ctg gat gac tgt gcc agc agc ccc tgc gac tcg ggc acc tgt ctg    1968
Asn Leu Asp Asp Cys Ala Ser Ser Pro Cys Asp Ser Gly Thr Cys Leu
                645                 650                 655

gac aag atc gat ggc tac gag tgt gcc tgt gag ccg ggc tac aca ggg    2016
Asp Lys Ile Asp Gly Tyr Glu Cys Ala Cys Glu Pro Gly Tyr Thr Gly
            660                 665                 670

agc atg tgt aac agc aac atc gat gag tgt gcg ggc aac ccc tgc cac    2064
Ser Met Cys Asn Ser Asn Ile Asp Glu Cys Ala Gly Asn Pro Cys His
        675                 680                 685

aac ggg ggc acc tgc gag gac ggc atc aat ggc ttc acc tgc cgc tgc    2112
Asn Gly Gly Thr Cys Glu Asp Gly Ile Asn Gly Phe Thr Cys Arg Cys
    690                 695                 700
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ccc gag ggc tac cac gac ccc acc tgc ctg tct gag gtc aat gag tgc 2160
Pro Glu Gly Tyr His Asp Pro Thr Cys Leu Ser Glu Val Asn Glu Cys
705 710 715 720

aac agc aac ccc tgc gtc cac ggg gcc tgc cgg gac agc ctc aac ggg 2208
Asn Ser Asn Pro Cys Val His Gly Ala Cys Arg Asp Ser Leu Asn Gly
725 730 735

tac aag tgc gac tgt gac cct ggg tgg agt ggg acc aac tgt gac atc 2256
Tyr Lys Cys Asp Cys Asp Pro Gly Trp Ser Gly Thr Asn Cys Asp Ile
740 745 750

aac aac aac gag tgt gaa tcc aac cct tgt gtc aac ggc ggc acc tgc 2304
Asn Asn Asn Glu Cys Glu Ser Asn Pro Cys Val Asn Gly Gly Thr Cys
755 760 765

aaa gac atg acc agt ggc atc gtg tgc acc tgc cgg gag ggc ttc agc 2352
Lys Asp Met Thr Ser Gly Ile Val Cys Thr Cys Arg Glu Gly Phe Ser
770 775 780

ggt ccc aac tgc cag acc aac atc aac gag tgt gcg tcc aac cca tgt 2400
Gly Pro Asn Cys Gln Thr Asn Ile Asn Glu Cys Ala Ser Asn Pro Cys
785 790 795 800

ctg aac aag ggc acg tgt att gac gac gtt gcc ggg tac aag tgc aac 2448
Leu Asn Lys Gly Thr Cys Ile Asp Asp Val Ala Gly Tyr Lys Cys Asn
805 810 815

tgc ctg ctg ccc tac aca ggt gcc acg tgt gag gtg gtg ctg gcc ccg 2496
Cys Leu Leu Pro Tyr Thr Gly Ala Thr Cys Glu Val Val Leu Ala Pro
820 825 830

tgt gcc ccc agc ccc tgc aga aac ggc ggg gag tgc agg caa tcc gag 2544
Cys Ala Pro Ser Pro Cys Arg Asn Gly Gly Glu Cys Arg Gln Ser Glu
835 840 845

gac tat gag agc ttc tcc tgt gtc tgc ccc acg gct ggg gcc aaa ggg 2592
Asp Tyr Glu Ser Phe Ser Cys Val Cys Pro Thr Ala Gly Ala Lys Gly
850 855 860

cag acc tgt gag gtc gac atc aac gag tgc gtt ctg agc ccg tgc cgg 2640
Gln Thr Cys Glu Val Asp Ile Asn Glu Cys Val Leu Ser Pro Cys Arg
865 870 875 880

cac ggc gca tcc tgc cag aac acc cac ggc gss tac cgc tgc cac tgc 2688
His Gly Ala Ser Cys Gln Asn Thr His Gly Xaa Tyr Arg Cys His Cys
885 890 895

cag gcc ggc tac agt ggg cgc aac tgc gag acc gac atc gac gac tgc 2736
Gln Ala Gly Tyr Ser Gly Arg Asn Cys Glu Thr Asp Ile Asp Asp Cys
900 905 910

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cgg ccc aac ccg tgt cac aac ggg ggc tcc tgc aca gac ggc atc aac    2784
Arg Pro Asn Pro Cys His Asn Gly Gly Ser Cys Thr Asp Gly Ile Asn
        915                 920                 925

acg gcc ttc tgc gac tgc ctg ccc ggc ttc cgg ggc act ttc tgt gag    2832
Thr Ala Phe Cys Asp Cys Leu Pro Gly Phe Arg Gly Thr Phe Cys Glu
    930                 935                 940

gag gac atc aac gag tgt gcc agt gac ccc tgc cgc aac ggg gcc aac    2880
Glu Asp Ile Asn Glu Cys Ala Ser Asp Pro Cys Arg Asn Gly Ala Asn
945                 950                 955                 960

tgc acg gac tgc gtg gac agc tac acg tgc acc tgc ccc gca ggc ttc    2928
Cys Thr Asp Cys Val Asp Ser Tyr Thr Cys Thr Cys Pro Ala Gly Phe
                965                 970                 975

agc ggg atc cac tgt gag aac aac acg cct gac tgc aca gag agc tcc    2976
Ser Gly Ile His Cys Glu Asn Asn Thr Pro Asp Cys Thr Glu Ser Ser
            980                 985                 990

tgc ttc aac ggt ggc acc tgc gtg gac ggc atc aac tcg ttc acc tgc    3024
Cys Phe Asn Gly Gly Thr Cys Val Asp Gly Ile Asn Ser Phe Thr Cys
        995                 1000                1005

ctg tgt cca ccc ggc ttc acg ggc agc tac tgc cag cac gta gtc aat    3072
Leu Cys Pro Pro Gly Phe Thr Gly Ser Tyr Cys Gln His Val Val Asn
    1010                1015                1020

gag tgc gac tca cga ccc tgc ctg cta ggc ggc acc tgt cag gac ggt    3120
Glu Cys Asp Ser Arg Pro Cys Leu Leu Gly Gly Thr Cys Gln Asp Gly
1025                1030                1035                1040

cgc ggt ctc cac agg tgc acc tgc ccc cag ggc tac act ggc ccc aac    3168
Arg Gly Leu His Arg Cys Thr Cys Pro Gln Gly Tyr Thr Gly Pro Asn
                1045                1050                1055

tgc cag aac ctt gtg cac tgg tgt gac tcc tcg ccc tgc aag aac ggc    3216
Cys Gln Asn Leu Val His Trp Cys Asp Ser Ser Pro Cys Lys Asn Gly
            1060                1065                1070

ggc aaa tgc tgg cag acc cac acc cag tac cgc tgc gag tgc ccc agc    3264
Gly Lys Cys Trp Gln Thr His Thr Gln Tyr Arg Cys Glu Cys Pro Ser
        1075                1080                1085

ggc tgg acc ggc ctt tac tgc gac gtg ccc agc gtg tcc tgt gag gtg    3312
Gly Trp Thr Gly Leu Tyr Cys Asp Val Pro Ser Val Ser Cys Glu Val
    1090                1095                1100

gct gcg cag cga caa ggt gtt gac gtt gcc cgc ctg tgc cag cat gga    3360
Ala Ala Gln Arg Gln Gly Val Asp Val Ala Arg Leu Cys Gln His Gly
1105                1110                1115                1120
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ggg ctc tgt gtg gac gcg ggc aac acg cac cac tgc cgc tgc cag gcg    3408
Gly Leu Cys Val Asp Ala Gly Asn Thr His His Cys Arg Cys Gln Ala
                1125                1130                1135

ggc tac aca ggc agc tac tgt gag gac ctg gtg gac gag tgc tca ccc    3456
Gly Tyr Thr Gly Ser Tyr Cys Glu Asp Leu Val Asp Glu Cys Ser Pro
            1140                1145                1150

agc ccc tgc cag aac ggg gcc acc tgc acg gac tac ctg ggc ggc tac    3504
Ser Pro Cys Gln Asn Gly Ala Thr Cys Thr Asp Tyr Leu Gly Gly Tyr
        1155                1160                1165

tcc tgc aag tgc gtg gcc ggc tac cac ggg gtg aac tgc tct gag gag    3552
Ser Cys Lys Cys Val Ala Gly Tyr His Gly Val Asn Cys Ser Glu Glu
    1170                1175                1180

atc gac gag tgc ctc tcc cac ccc tgc cag aac ggg ggc acc tgc ctc    3600
Ile Asp Glu Cys Leu Ser His Pro Cys Gln Asn Gly Gly Thr Cys Leu
1185                1190                1195                1200

gac ctc ccc aac acc tac aag tgc tcc tgc cca cgg ggc act cag ggt    3648
Asp Leu Pro Asn Thr Tyr Lys Cys Ser Cys Pro Arg Gly Thr Gln Gly
                1205                1210                1215

gtg cac tgt gag atc aac gtg gac gac tgc aat ccc ccc gtt gac ccc    3696
Val His Cys Glu Ile Asn Val Asp Asp Cys Asn Pro Pro Val Asp Pro
            1220                1225                1230

gtg tcc cgg agc ccc aag tgc ttt aac aac ggc acc tgc gtg gac cag    3744
Val Ser Arg Ser Pro Lys Cys Phe Asn Asn Gly Thr Cys Val Asp Gln
        1235                1240                1245

gtg ggc ggc tac agc tgc acc tgc ccg ccg ggc ttc gtg ggt gag cgc    3792
Val Gly Gly Tyr Ser Cys Thr Cys Pro Pro Gly Phe Val Gly Glu Arg
    1250                1255                1260

tgt gag ggg gat gtc aac gag tgc ctg tcc aat ccc tgc gac gcc cgt    3840
Cys Glu Gly Asp Val Asn Glu Cys Leu Ser Asn Pro Cys Asp Ala Arg
1265                1270                1275                1280

ggc acc cag aac tgc gtg cag cgc gtc aat gac ttc cac tgc gag tgc    3888
Gly Thr Gln Asn Cys Val Gln Arg Val Asn Asp Phe His Cys Glu Cys
                1285                1290                1295

cgt gct ggt cac acc ggg cgc cgc tgc gag tcc gtc atc aat ggc tgc    3936
Arg Ala Gly His Thr Gly Arg Arg Cys Glu Ser Val Ile Asn Gly Cys
            1300                1305                1310

aaa ggc aag ccc tgc aag aat ggg ggc acc tgc gcc gtg gcc tcc aac    3984
Lys Gly Lys Pro Cys Lys Asn Gly Gly Thr Cys Ala Val Ala Ser Asn
        1315                1320                1325
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acc gcc cgc ggg ttc atc tgc aag tgc cct gcg ggc ttc gag ggc gcc     4032
Thr Ala Arg Gly Phe Ile Cys Lys Cys Pro Ala Gly Phe Glu Gly Ala
    1330                1335                1340

acg tgt gag aat gac gct cgt acc tgc ggc agc ctg cgc tgc ctc aac     4080
Thr Cys Glu Asn Asp Ala Arg Thr Cys Gly Ser Leu Arg Cys Leu Asn
1345                1350                1355                1360

ggc ggc aca tgc atc tcc ggc ccg cgc agc ccc acc tgc ctg tgc ctg     4128
Gly Gly Thr Cys Ile Ser Gly Pro Arg Ser Pro Thr Cys Leu Cys Leu
            1365                1370                1375

ggc ccc ttc acg ggc ccc gaa tgc cag ttc ccg gcc agc agc ccc tgc     4176
Gly Pro Phe Thr Gly Pro Glu Cys Gln Phe Pro Ala Ser Ser Pro Cys
        1380                1385                1390

ctg ggc ggc aac ccc tgc tac aac cag ggg acc tgt gag ccc aca tcc     4224
Leu Gly Gly Asn Pro Cys Tyr Asn Gln Gly Thr Cys Glu Pro Thr Ser
    1395                1400                1405

gag agc ccc ttc tac cgt tgc ctg tgc ccc gcc aaa ttc aac ggg ctc     4272
Glu Ser Pro Phe Tyr Arg Cys Leu Cys Pro Ala Lys Phe Asn Gly Leu
    1410                1415                1420

ttg tgc cac atc ctg gac tac agc ttc ggg ggt ggg gcc ggg cgc gac     4320
Leu Cys His Ile Leu Asp Tyr Ser Phe Gly Gly Gly Ala Gly Arg Asp
1425                1430                1435                1440

atc ccc ccg ccg ctg atc gag gag gcg tgc gag ctg ccc gag tgc cag     4368
Ile Pro Pro Pro Leu Ile Glu Glu Ala Cys Glu Leu Pro Glu Cys Gln
            1445                1450                1455

gag gac gcg ggc aac aag gtc tgc agc ctg cag tgc aac aac cac gcg     4416
Glu Asp Ala Gly Asn Lys Val Cys Ser Leu Gln Cys Asn Asn His Ala
        1460                1465                1470

tgc ggc tgg gac ggc ggt gac tgc tcc ctc aac ttc aat gac ccc tgg     4464
Cys Gly Trp Asp Gly Gly Asp Cys Ser Leu Asn Phe Asn Asp Pro Trp
    1475                1480                1485

aag aac tgc acg cag tct ctg cag tgc tgg aag tac ttc agt gac ggc     4512
Lys Asn Cys Thr Gln Ser Leu Gln Cys Trp Lys Tyr Phe Ser Asp Gly
    1490                1495                1500

cac tgt gac agc cag tgc aac tca gcc ggc tgc ctc ttc gac ggc ttt     4560
His Cys Asp Ser Gln Cys Asn Ser Ala Gly Cys Leu Phe Asp Gly Phe
1505                1510                1515                1520

gac tgc cag cgt gcg gaa ggc cag tgc aac ccc ctg tac gac cag tac     4608
Asp Cys Gln Arg Ala Glu Gly Gln Cys Asn Pro Leu Tyr Asp Gln Tyr
            1525                1530                1535
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tgc aag gac cac ttc agc gac ggg cac tgc gac cag ggc tgc aac agc    4656
Cys Lys Asp His Phe Ser Asp Gly His Cys Asp Gln Gly Cys Asn Ser
            1540                1545                1550

gcg gag tgc gag tgg gac ggg ctg gac tgt gcg gag cat gta ccc gag    4704
Ala Glu Cys Glu Trp Asp Gly Leu Asp Cys Ala Glu His Val Pro Glu
        1555                1560                1565

agg ctg gcg gcc ggc acg ctg gtg gtg gtg gtg ctg atg ccg ccg gag    4752
Arg Leu Ala Ala Gly Thr Leu Val Val Val Val Leu Met Pro Pro Glu
    1570                1575                1580

cag ctg cgc aac agc tcc ttc cac ttc ctg cgg gag ctc agc cgc gtg    4800
Gln Leu Arg Asn Ser Ser Phe His Phe Leu Arg Glu Leu Ser Arg Val
1585                1590                1595                1600

ctg cac acc aac gtg gtc ttc aag cgt gac gca cac ggc cag cag atg    4848
Leu His Thr Asn Val Val Phe Lys Arg Asp Ala His Gly Gln Gln Met
                1605                1610                1615

atc ttc ccc tac tac ggc cgc gag gag gag ctg cgc aag cac ccc atc    4896
Ile Phe Pro Tyr Tyr Gly Arg Glu Glu Glu Leu Arg Lys His Pro Ile
            1620                1625                1630

aag cgt gcc gcc gag ggc tgg gcc gca cct gac gcc ctg ctg ggc cag    4944
Lys Arg Ala Ala Glu Gly Trp Ala Ala Pro Asp Ala Leu Leu Gly Gln
        1635                1640                1645

gtg aag gcc tcg ctg ctc cct ggt ggc agc gag ggt ggg cgg cgg cgg    4992
Val Lys Ala Ser Leu Leu Pro Gly Gly Ser Glu Gly Gly Arg Arg Arg
    1650                1655                1660

agg gag ctg gac ccc atg gac gtc cgc ggc tcc atc gtc tac ctg gag    5040
Arg Glu Leu Asp Pro Met Asp Val Arg Gly Ser Ile Val Tyr Leu Glu
1665                1670                1675                1680

att gac aac cgg cag tgt gtg cag gcc tcc tcg cag tgc ttc cag agt    5088
Ile Asp Asn Arg Gln Cys Val Gln Ala Ser Ser Gln Cys Phe Gln Ser
                1685                1690                1695

gcc acc gac gtg gcc gca ttc ctg gga gcg ctc gcc tcg ctg ggc agc    5136
Ala Thr Asp Val Ala Ala Phe Leu Gly Ala Leu Ala Ser Leu Gly Ser
            1700                1705                1710

ctc aac atc ccc tac aag atc gag gcc gtg cag agt gag acc gtg gag    5184
Leu Asn Ile Pro Tyr Lys Ile Glu Ala Val Gln Ser Glu Thr Val Glu
        1715                1720                1725

ccg ccc ccg ccg gcg cag ctg cac ttc atg tac gtg gcg gcg gcc gcc    5232
Pro Pro Pro Pro Ala Gln Leu His Phe Met Tyr Val Ala Ala Ala Ala
    1730                1735                1740
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ttt gtg ctt ctg ttc ttc gtg ggc tgc ggg gtg ctg ctg tcc cgc aag 5280
Phe Val Leu Leu Phe Phe Val Gly Cys Gly Val Leu Leu Ser Arg Lys
1745 1750 1755 1760

cgc cgg cgg cag cat ggc cag ctc tgg ttc cct gag ggc ttc aaa gtg 5328
Arg Arg Arg Gln His Gly Gln Leu Trp Phe Pro Glu Gly Phe Lys Val
1765 1770 1775

tct gag gcc agc aag aag aag cgg cgg gag ccc ctc ggc gag gac tcc 5376
Ser Glu Ala Ser Lys Lys Lys Arg Arg Glu Pro Leu Gly Glu Asp Ser
1780 1785 1790

gtg ggc ctc aag ccc ctg aag aac gct tca gac ggt gcc ctc atg gac 5424
Val Gly Leu Lys Pro Leu Lys Asn Ala Ser Asp Gly Ala Leu Met Asp
1795 1800 1805

gac aac cag aat gag tgg ggg gac gag gac ctg gag acc aag aag ttc 5472
Asp Asn Gln Asn Glu Trp Gly Asp Glu Asp Leu Glu Thr Lys Lys Phe
1810 1815 1820

cgg ttc gag gag ccc gtg gtt ctg cct gac ctg gac gac cag aca gac 5520
Arg Phe Glu Glu Pro Val Val Leu Pro Asp Leu Asp Asp Gln Thr Asp
1825 1830 1835 1840

cac cgg cag tgg act cag cag cac ctg gat gcc gct gac ctg cgc atg 5568
His Arg Gln Trp Thr Gln Gln His Leu Asp Ala Ala Asp Leu Arg Met
1845 1850 1855

tct gcc atg gcc ccc aca ccg ccc cag ggt gag gtt gac gcc gac tgc 5616
Ser Ala Met Ala Pro Thr Pro Pro Gln Gly Glu Val Asp Ala Asp Cys
1860 1865 1870

atg gac gtc aat gtc cgc ggg cct gat ggc ttc acc ccg ctc atg atc 5664
Met Asp Val Asn Val Arg Gly Pro Asp Gly Phe Thr Pro Leu Met Ile
1875 1880 1885

gcc tcc tgc agc ggg ggc ggc ctg gag acg ggc aac agc gag gaa gag 5712
Ala Ser Cys Ser Gly Gly Gly Leu Glu Thr Gly Asn Ser Glu Glu Glu
1890 1895 1900

gag gac gcg ccg gcc gtc atc tcc gac ttc atc tac cag ggc gcc agc 5760
Glu Asp Ala Pro Ala Val Ile Ser Asp Phe Ile Tyr Gln Gly Ala Ser
1905 1910 1915 1920

ctg cac aac cag aca gac cgc acg ggc gag acc gcc ttg cac ctg gcc 5808
Leu His Asn Gln Thr Asp Arg Thr Gly Glu Thr Ala Leu His Leu Ala
1925 1930 1935

gcc cgc tac tca cgc tct gat gcc gcc aag cgc ctg ctg gag gcc agc 5856
Ala Arg Tyr Ser Arg Ser Asp Ala Ala Lys Arg Leu Leu Glu Ala Ser
1940 1945 1950

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gca gat gcc aac atc cag gac aac atg ggc cgc acc ccg ctg cat gcg     5904
Ala Asp Ala Asn Ile Gln Asp Asn Met Gly Arg Thr Pro Leu His Ala
        1955                1960                1965

gct gtg tct gcc gac gca caa ggt gtc ttc cag atc ctg atc cgg aac     5952
Ala Val Ser Ala Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn
    1970                1975                1980

cga gcc aca gac ctg gat gcc cgc atg cat gat ggc acg acg cca ctg     6000
Arg Ala Thr Asp Leu Asp Ala Arg Met His Asp Gly Thr Thr Pro Leu
1985                1990                1995                2000

atc ctg gct gcc cgc ctg gcc gtg gag ggc atg ctg gag gac ctc atc     6048
Ile Leu Ala Ala Arg Leu Ala Val Glu Gly Met Leu Glu Asp Leu Ile
                2005                2010                2015

aac tca cac gcc gac gtc aac gcc gta gat gac ctg ggc aag tcc gcc     6096
Asn Ser His Ala Asp Val Asn Ala Val Asp Asp Leu Gly Lys Ser Ala
            2020                2025                2030

ctg cac tgg gcc gcc gcc gtg aac aat gtg gat gcc gca gtt gtg ctc     6144
Leu His Trp Ala Ala Ala Val Asn Asn Val Asp Ala Ala Val Val Leu
        2035                2040                2045

ctg aag aac ggg gct aac aaa gat atg cag aac aac agg gag gag aca     6192
Leu Lys Asn Gly Ala Asn Lys Asp Met Gln Asn Asn Arg Glu Glu Thr
    2050                2055                2060

ccc ctg ttt ctg gcc gcc cgg gag ggc agc tac gag acc gcc aag gtg     6240
Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala Lys Val
2065                2070                2075                2080

ctg ctg gac cac ttt gcc aac cgg gac atc acg gat cat atg gac cgc     6288
Leu Leu Asp His Phe Ala Asn Arg Asp Ile Thr Asp His Met Asp Arg
                2085                2090                2095

ctg ccg cgc gac atc gca cag gag cgc atg cat cac gac atc gtg agg     6336
Leu Pro Arg Asp Ile Ala Gln Glu Arg Met His His Asp Ile Val Arg
            2100                2105                2110

ctg ctg gac gag tac aac ctg gtg cgc agc ccg cag ctg cac gga gcc     6384
Leu Leu Asp Glu Tyr Asn Leu Val Arg Ser Pro Gln Leu His Gly Ala
        2115                2120                2125

ccg ctg ggg ggc acg ccc acc ctg tcg ccc ccg ctc tgc tcg ccc aac     6432
Pro Leu Gly Gly Thr Pro Thr Leu Ser Pro Pro Leu Cys Ser Pro Asn
    2130                2135                2140

ggc tac ctg ggc agc ctc aag ccc ggc gtg cag ggc aag aag gtc cgc     6480
Gly Tyr Leu Gly Ser Leu Lys Pro Gly Val Gln Gly Lys Lys Val Arg
2145                2150                2155                2160
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aag ccc agc agc aaa ggc ctg gcc tgt gga agc aag gag gcc aag gac     6528
Lys Pro Ser Ser Lys Gly Leu Ala Cys Gly Ser Lys Glu Ala Lys Asp
                2165                2170                2175

ctc aag gca cgg agg aag aag tcc cag gat ggc aag ggc tgc ctg ctg     6576
Leu Lys Ala Arg Arg Lys Lys Ser Gln Asp Gly Lys Gly Cys Leu Leu
            2180                2185                2190

gac agc tcc ggc atg ctc tcg ccc gtg gac tcc ctg gag tca ccc cat     6624
Asp Ser Ser Gly Met Leu Ser Pro Val Asp Ser Leu Glu Ser Pro His
        2195                2200                2205

ggc tac ctg tca gac gtg gcc tcg ccg cca ctg ctg ccc tcc ccg ttc     6672
Gly Tyr Leu Ser Asp Val Ala Ser Pro Pro Leu Leu Pro Ser Pro Phe
    2210                2215                2220

cag cag tct ccg tcc gtg ccc ctc aac cac ctg cct ggg atg ccc gac     6720
Gln Gln Ser Pro Ser Val Pro Leu Asn His Leu Pro Gly Met Pro Asp
2225                2230                2235                2240

acc cac ctg ggc atc ggg cac ctg aac gtg gcg gcc aag ccc gag atg     6768
Thr His Leu Gly Ile Gly His Leu Asn Val Ala Ala Lys Pro Glu Met
                2245                2250                2255

gcg gcg ctg ggt ggg ggc ggc cgg ctg gcc ttt gag act ggc cca cct     6816
Ala Ala Leu Gly Gly Gly Gly Arg Leu Ala Phe Glu Thr Gly Pro Pro
            2260                2265                2270

cgt ctc tcc cac ctg cct gtg gcc tct ggc acc agc acc gtc ctg ggc     6864
Arg Leu Ser His Leu Pro Val Ala Ser Gly Thr Ser Thr Val Leu Gly
        2275                2280                2285

tcc agc agc gga ggg gcc ctg aat ttc act gtg ggc ggg tcc acc agt     6912
Ser Ser Ser Gly Gly Ala Leu Asn Phe Thr Val Gly Gly Ser Thr Ser
    2290                2295                2300

ttg aat ggt caa tgc gag tgg ctg tcc cgg ctg cag agc ggc atg gtg     6960
Leu Asn Gly Gln Cys Glu Trp Leu Ser Arg Leu Gln Ser Gly Met Val
2305                2310                2315                2320

ccg aac caa tac aac cct ctg cgg ggg agt gtg gca cca ggc ccc ctg     7008
Pro Asn Gln Tyr Asn Pro Leu Arg Gly Ser Val Ala Pro Gly Pro Leu
                2325                2330                2335

agc aca cag gcc ccc tcc ctg cag cat ggc atg gta ggc ccg ctg cac     7056
Ser Thr Gln Ala Pro Ser Leu Gln His Gly Met Val Gly Pro Leu His
            2340                2345                2350

agt agc ctt gct gcc agc gcc ctg tcc cag atg atg agc tac cag ggc     7104
Ser Ser Leu Ala Ala Ser Ala Leu Ser Gln Met Met Ser Tyr Gln Gly
        2355                2360                2365
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ctg ccc agc acc cgg ctg gcc acc cag cct cac ctg gtg cag acc cag      7152
Leu Pro Ser Thr Arg Leu Ala Thr Gln Pro His Leu Val Gln Thr Gln
    2370                2375                2380

cag gtg cag cca caa aac tta cag atg cag cag cag aac ctg cag cca      7200
Gln Val Gln Pro Gln Asn Leu Gln Met Gln Gln Gln Asn Leu Gln Pro
2385                2390                2395                2400

gca aac atc cag cag cag caa agc ctg cag ccg cca cca cca cca cca      7248
Ala Asn Ile Gln Gln Gln Gln Ser Leu Gln Pro Pro Pro Pro Pro Pro
                2405                2410                2415

cag ccg cac ctt ggc gtg agc tca gca gcc agc ggc cac ctg ggc cgg      7296
Gln Pro His Leu Gly Val Ser Ser Ala Ala Ser Gly His Leu Gly Arg
            2420                2425                2430

agc ttc ctg agt gga gag ccg agc cag gca gac gtg cag cca ctg ggc      7344
Ser Phe Leu Ser Gly Glu Pro Ser Gln Ala Asp Val Gln Pro Leu Gly
        2435                2440                2445

ccc agc agc ctg gcg gtg cac act att ctg ccc cag gag agc ccc gcc      7392
Pro Ser Ser Leu Ala Val His Thr Ile Leu Pro Gln Glu Ser Pro Ala
    2450                2455                2460

ctg ccc acg tcg ctg cca tcc tcg ctg gtc cca ccc gtg acc gca gcc      7440
Leu Pro Thr Ser Leu Pro Ser Ser Leu Val Pro Pro Val Thr Ala Ala
2465                2470                2475                2480

cag ttc ctg acg ccc ccc tcg cag cac agc tac tcc tcg cct gtg gac      7488
Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro Val Asp
                2485                2490                2495

aac acc ccc agc cac cag cta cag gtg cct gag cac ccc ttc ctg acc      7536
Asn Thr Pro Ser His Gln Leu Gln Val Pro Glu His Pro Phe Leu Thr
            2500                2505                2510

cct tcg ccg gag tcg ccc gac caa tgg tcg tcc tcg tcg ccg cac tct      7584
Pro Ser Pro Glu Ser Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser
        2515                2520                2525

aat gtg tct gac tgg tct gag ggc gtg tcg tcg ccc ccg acc tcc atg      7632
Asn Val Ser Asp Trp Ser Glu Gly Val Ser Ser Pro Pro Thr Ser Met
    2530                2535                2540

cag tcc cag atc gcg cgc atc ccg gag gcg ttc aag taa                  7671
Gln Ser Gln Ile Ala Arg Ile Pro Glu Ala Phe Lys
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Asn Gly Gly Lys Cys Glu Ala Ala Asn Gly Thr Glu Ala Cys Val Cys
35 40 45
Gly Gly Ala Phe Val Gly Pro Arg Cys Gln Asp Pro Asn Pro Cys Leu
50 55 60
Ser Thr Pro Cys Lys Asn Ala Gly Thr Cys His Val Val Asp Arg Arg
65 70 75 80
Gly Val Ala Asp Tyr Ala Cys Ser Cys Ala Leu Gly Phe Ser Gly Pro
85 90 95
Leu Cys Leu Thr Pro Leu Asp Asn Ala Cys Leu Thr Asn Pro Cys Arg
100 105 110
Asn Gly Gly Thr Cys Asp Leu Leu Thr Leu Thr Glu Tyr Lys Cys Arg
115 120 125
Cys Pro Pro Gly Trp Ser Gly Lys Ser Cys Gln Gln Ala Asp Pro Cys
130 135 140
Ala Ser Asn Pro Cys Ala Asn Gly Gly Gln Cys Leu Pro Phe Glu Ala
145 150 155 160
Ser Tyr Ile Cys His Cys Pro Pro Ser Phe His Gly Pro Thr Cys Arg
165 170 175
Gln Asp Val Asn Glu Cys Gly Gln Lys Pro Arg Leu Cys Arg His Gly
180 185 190
Gly Thr Cys His Asn Glu Val Gly Ser Tyr Arg Cys Val Cys Arg Ala
195 200 205
Thr His Thr Gly Pro Asn Cys Glu Arg Pro Tyr Val Pro Cys Ser Pro
210 215 220
Ser Pro Cys Gln Asn Gly Gly Thr Cys Arg Pro Thr Gly Asp Val Thr
225 230 235 240
His Glu Cys Ala Cys Leu Pro Gly Phe Thr Gly Gln Asn Cys Glu Glu
245 250 255
Asn Ile Asp Asp Cys Pro Gly Asn Asn Cys Lys Asn Gly Gly Ala Cys
260 265 270
Val Asp Gly Val Asn Thr Tyr Asn Cys Pro Cys Pro Pro Glu Trp Thr
275 280 285
Gly Gln Tyr Cys Thr Glu Asp Val Asp Glu Cys Gln Leu Met Pro Asn
290 295 300
Ala Cys Gln Asn Gly Gly Thr Cys His Asn Thr His Gly Gly Tyr Asn

305 310 315 320
Cys Val Cys Val Asn Gly Trp Thr Gly Glu Asp Cys Ser Glu Asn Ile
325 330 335
Asp Asp Cys Ala Ser Ala Ala Cys Phe His Gly Ala Thr Cys His Asp
340 345 350
Arg Val Ala Ser Phe Tyr Cys Glu Cys Pro His Gly Arg Thr Gly Leu
355 360 365
Leu Cys His Leu Asn Asp Ala Cys Ile Ser Asn Pro Cys Asn Glu Gly
370 375 380
Ser Asn Cys Asp Thr Asn Pro Val Asn Gly Lys Ala Ile Cys Thr Cys
385 390 395 400
Pro Ser Gly Tyr Thr Gly Pro Ala Cys Ser Gln Asp Val Asp Glu Cys
405 410 415
Ser Leu Gly Ala Asn Pro Cys Glu His Ala Gly Lys Cys Ile Asn Thr
420 425 430
Leu Gly Ser Phe Glu Cys Gln Cys Leu Gln Gly Tyr Thr Gly Pro Arg
435 440 445
Cys Glu Ile Asp Val Asn Glu Cys Val Ser Asn Pro Cys Gln Asn Asp
450 455 460
Ala Thr Cys Leu Asp Gln Ile Gly Glu Phe Gln Cys Met Cys Met Pro
465 470 475 480
Gly Tyr Glu Gly Val His Cys Glu Val Asn Thr Asp Glu Cys Ala Ser
485 490 495
Ser Pro Cys Leu His Asn Gly Arg Cys Leu Asp Lys Ile Asn Glu Phe
500 505 510
Gln Cys Glu Cys Pro Thr Gly Phe Thr Gly His Leu Cys Gln Tyr Asp
515 520 525
Val Asp Glu Cys Ala Ser Thr Pro Cys Lys Asn Gly Ala Lys Cys Leu
530 535 540
Asp Gly Pro Asn Thr Tyr Thr Cys Val Cys Thr Glu Gly Tyr Thr Gly
545 550 555 560
Thr His Cys Glu Val Asp Ile Asp Glu Cys Asp Pro Asp Pro Cys His
565 570 575
Tyr Gly Ser Cys Lys Asp Gly Val Ala Thr Phe Thr Cys Leu Cys Arg
580 585 590
Pro Gly Tyr Thr Gly His His Cys Glu Thr Asn Ile Asn Glu Cys Ser
595 600 605
Ser Gln Pro Cys Arg Leu Arg Gly Thr Cys Gln Asp Pro Asp Asn Ala
610 615 620
Tyr Leu Cys Phe Cys Leu Lys Gly Thr Thr Gly Pro Asn Cys Glu Ile
625 630 635 640
Asn Leu Asp Asp Cys Ala Ser Ser Pro Cys Asp Ser Gly Thr Cys Leu
645 650 655
Asp Lys Ile Asp Gly Tyr Glu Cys Ala Cys Glu Pro Gly Tyr Thr Gly
660 665 670
Ser Met Cys Asn Ser Asn Ile Asp Glu Cys Ala Gly Asn Pro Cys His
675 680 685
Asn Gly Gly Thr Cys Glu Asp Gly Ile Asn Gly Phe Thr Cys Arg Cys
690 695 700
Pro Glu Gly Tyr His Asp Pro Thr Cys Leu Ser Glu Val Asn Glu Cys
705 710 715 720
Asn Ser Asn Pro Cys Val His Gly Ala Cys Arg Asp Ser Leu Asn Gly

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                725                 730                 735
Tyr Lys Cys Asp Cys Asp Pro Gly Trp Ser Gly Thr Asn Cys Asp Ile
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Asn Asn Asn Glu Cys Glu Ser Asn Pro Cys Val Asn Gly Gly Thr Cys
        755                 760                 765
Lys Asp Met Thr Ser Gly Ile Val Cys Thr Cys Arg Glu Gly Phe Ser
    770                 775                 780
Gly Pro Asn Cys Gln Thr Asn Ile Asn Glu Cys Ala Ser Asn Pro Cys
785                 790                 795                 800
Leu Asn Lys Gly Thr Cys Ile Asp Asp Val Ala Gly Tyr Lys Cys Asn
                805                 810                 815
Cys Leu Leu Pro Tyr Thr Gly Ala Thr Cys Glu Val Val Leu Ala Pro
            820                 825                 830
Cys Ala Pro Ser Pro Cys Arg Asn Gly Gly Glu Cys Arg Gln Ser Glu
        835                 840                 845
Asp Tyr Glu Ser Phe Ser Cys Val Cys Pro Thr Ala Gly Ala Lys Gly
    850                 855                 860
Gln Thr Cys Glu Val Asp Ile Asn Glu Cys Val Leu Ser Pro Cys Arg
865                 870                 875                 880
His Gly Ala Ser Cys Gln Asn Thr His Gly Xaa Tyr Arg Cys His Cys
                885                 890                 895
Gln Ala Gly Tyr Ser Gly Arg Asn Cys Glu Thr Asp Ile Asp Asp Cys
            900                 905                 910
Arg Pro Asn Pro Cys His Asn Gly Gly Ser Cys Thr Asp Gly Ile Asn
        915                 920                 925
Thr Ala Phe Cys Asp Cys Leu Pro Gly Phe Arg Gly Thr Phe Cys Glu
    930                 935                 940
Glu Asp Ile Asn Glu Cys Ala Ser Asp Pro Cys Arg Asn Gly Ala Asn
945                 950                 955                 960
Cys Thr Asp Cys Val Asp Ser Tyr Thr Cys Thr Cys Pro Ala Gly Phe
                965                 970                 975
Ser Gly Ile His Cys Glu Asn Asn Thr Pro Asp Cys Thr Glu Ser Ser
            980                 985                 990
Cys Phe Asn Gly Gly Thr Cys Val Asp Gly Ile Asn Ser Phe Thr Cys
        995                 1000                1005
Leu Cys Pro Pro Gly Phe Thr Gly Ser Tyr Cys Gln His Val Val Asn
    1010                1015                1020
Glu Cys Asp Ser Arg Pro Cys Leu Leu Gly Gly Thr Cys Gln Asp Gly
1025                1030                1035                1040
Arg Gly Leu His Arg Cys Thr Cys Pro Gln Gly Tyr Thr Gly Pro Asn
                1045                1050                1055
Cys Gln Asn Leu Val His Trp Cys Asp Ser Ser Pro Cys Lys Asn Gly
            1060                1065                1070
Gly Lys Cys Trp Gln Thr His Thr Gln Tyr Arg Cys Glu Cys Pro Ser
        1075                1080                1085
Gly Trp Thr Gly Leu Tyr Cys Asp Val Pro Ser Val Ser Cys Glu Val
    1090                1095                1100
Ala Ala Gln Arg Gln Gly Val Asp Val Ala Arg Leu Cys Gln His Gly
1105                1110                1115                1120
Gly Leu Cys Val Asp Ala Gly Asn Thr His His Cys Arg Cys Gln Ala
                1125                1130                1135
Gly Tyr Thr Gly Ser Tyr Cys Glu Asp Leu Val Asp Glu Cys Ser Pro
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1140 1145 1150
Ser Pro Cys Gln Asn Gly Ala Thr Cys Thr Asp Tyr Leu Gly Gly Tyr
1155 1160 1165
Ser Cys Lys Cys Val Ala Gly Tyr His Gly Val Asn Cys Ser Glu Glu
1170 1175 1180
Ile Asp Glu Cys Leu Ser His Pro Cys Gln Asn Gly Gly Thr Cys Leu
1185 1190 1195 1200
Asp Leu Pro Asn Thr Tyr Lys Cys Ser Cys Pro Arg Gly Thr Gln Gly
1205 1210 1215
Val His Cys Glu Ile Asn Val Asp Asp Cys Asn Pro Pro Val Asp Pro
1220 1225 1230
Val Ser Arg Ser Pro Lys Cys Phe Asn Asn Gly Thr Cys Val Asp Gln
1235 1240 1245
Val Gly Gly Tyr Ser Cys Thr Cys Pro Pro Gly Phe Val Gly Glu Arg
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Cys Glu Gly Asp Val Asn Glu Cys Leu Ser Asn Pro Cys Asp Ala Arg
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Gly Thr Gln Asn Cys Val Gln Arg Val Asn Asp Phe His Cys Glu Cys
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Lys Gly Lys Pro Cys Lys Asn Gly Gly Thr Cys Ala Val Ala Ser Asn
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Thr Cys Glu Asn Asp Ala Arg Thr Cys Gly Ser Leu Arg Cys Leu Asn
1345 1350 1355 1360
Gly Gly Thr Cys Ile Ser Gly Pro Arg Ser Pro Thr Cys Leu Cys Leu
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Gly Pro Phe Thr Gly Pro Glu Cys Gln Phe Pro Ala Ser Ser Pro Cys
1380 1385 1390
Leu Gly Gly Asn Pro Cys Tyr Asn Gln Gly Thr Cys Glu Pro Thr Ser
1395 1400 1405
Glu Ser Pro Phe Tyr Arg Cys Leu Cys Pro Ala Lys Phe Asn Gly Leu
1410 1415 1420
Leu Cys His Ile Leu Asp Tyr Ser Phe Gly Gly Gly Ala Gly Arg Asp
1425 1430 1435 1440
Ile Pro Pro Pro Leu Ile Glu Glu Ala Cys Glu Leu Pro Glu Cys Gln
1445 1450 1455
Glu Asp Ala Gly Asn Lys Val Cys Ser Leu Gln Cys Asn Asn His Ala
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Cys Gly Trp Asp Gly Gly Asp Cys Ser Leu Asn Phe Asn Asp Pro Trp
1475 1480 1485
Lys Asn Cys Thr Gln Ser Leu Gln Cys Trp Lys Tyr Phe Ser Asp Gly
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His Cys Asp Ser Gln Cys Asn Ser Ala Gly Cys Leu Phe Asp Gly Phe
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1525 1530 1535
Cys Lys Asp His Phe Ser Asp Gly His Cys Asp Gln Gly Cys Asn Ser
1540 1545 1550
Ala Glu Cys Glu Trp Asp Gly Leu Asp Cys Ala Glu His Val Pro Glu

1555 1560 1565
Arg Leu Ala Ala Gly Thr Leu Val Val Val Val Leu Met Pro Pro Glu
1570 1575 1580
Gln Leu Arg Asn Ser Ser Phe His Phe Leu Arg Glu Leu Ser Arg Val
1585 1590 1595 1600
Leu His Thr Asn Val Val Phe Lys Arg Asp Ala His Gly Gln Gln Met
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Ile Phe Pro Tyr Tyr Gly Arg Glu Glu Glu Leu Arg Lys His Pro Ile
1620 1625 1630
Lys Arg Ala Ala Glu Gly Trp Ala Ala Pro Asp Ala Leu Leu Gly Gln
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Val Lys Ala Ser Leu Leu Pro Gly Gly Ser Glu Gly Gly Arg Arg Arg
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Arg Glu Leu Asp Pro Met Asp Val Arg Gly Ser Ile Val Tyr Leu Glu
1665 1670 1675 1680
Ile Asp Asn Arg Gln Cys Val Gln Ala Ser Ser Gln Cys Phe Gln Ser
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Ala Thr Asp Val Ala Ala Phe Leu Gly Ala Leu Ala Ser Leu Gly Ser
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1715 1720 1725
Pro Pro Pro Pro Ala Gln Leu His Phe Met Tyr Val Ala Ala Ala Ala
1730 1735 1740
Phe Val Leu Leu Phe Phe Val Gly Cys Gly Val Leu Leu Ser Arg Lys
1745 1750 1755 1760
Arg Arg Arg Gln His Gly Gln Leu Trp Phe Pro Glu Gly Phe Lys Val
1765 1770 1775
Ser Glu Ala Ser Lys Lys Lys Arg Arg Glu Pro Leu Gly Glu Asp Ser
1780 1785 1790
Val Gly Leu Lys Pro Leu Lys Asn Ala Ser Asp Gly Ala Leu Met Asp
1795 1800 1805
Asp Asn Gln Asn Glu Trp Gly Asp Glu Asp Leu Glu Thr Lys Lys Phe
1810 1815 1820
Arg Phe Glu Glu Pro Val Val Leu Pro Asp Leu Asp Asp Gln Thr Asp
1825 1830 1835 1840
His Arg Gln Trp Thr Gln Gln His Leu Asp Ala Ala Asp Leu Arg Met
1845 1850 1855
Ser Ala Met Ala Pro Thr Pro Pro Gln Gly Glu Val Asp Ala Asp Cys
1860 1865 1870
Met Asp Val Asn Val Arg Gly Pro Asp Gly Phe Thr Pro Leu Met Ile
1875 1880 1885
Ala Ser Cys Ser Gly Gly Gly Leu Glu Thr Gly Asn Ser Glu Glu Glu
1890 1895 1900
Glu Asp Ala Pro Ala Val Ile Ser Asp Phe Ile Tyr Gln Gly Ala Ser
1905 1910 1915 1920
Leu His Asn Gln Thr Asp Arg Thr Gly Glu Thr Ala Leu His Leu Ala
1925 1930 1935
Ala Arg Tyr Ser Arg Ser Asp Ala Ala Lys Arg Leu Leu Glu Ala Ser
1940 1945 1950
Ala Asp Ala Asn Ile Gln Asp Asn Met Gly Arg Thr Pro Leu His Ala
1955 1960 1965
Ala Val Ser Ala Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn

1970 1975 1980
Arg Ala Thr Asp Leu Asp Ala Arg Met His Asp Gly Thr Thr Pro Leu
1985 1990 1995 2000
Ile Leu Ala Ala Arg Leu Ala Val Glu Gly Met Leu Glu Asp Leu Ile
2005 2010 2015
Asn Ser His Ala Asp Val Asn Ala Val Asp Asp Leu Gly Lys Ser Ala
2020 2025 2030
Leu His Trp Ala Ala Ala Val Asn Asn Val Asp Ala Ala Val Val Leu
2035 2040 2045
Leu Lys Asn Gly Ala Asn Lys Asp Met Gln Asn Asn Arg Glu Glu Thr
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Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala Lys Val
2065 2070 2075 2080
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Leu Pro Arg Asp Ile Ala Gln Glu Arg Met His His Asp Ile Val Arg
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2115 2120 2125
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2355 2360 2365
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Ser Phe Leu Ser Gly Glu Pro Ser Gln Ala Asp Val Gln Pro Leu Gly
        2435                2440                2445
Pro Ser Ser Leu Ala Val His Thr Ile Leu Pro Gln Glu Ser Pro Ala
    2450                2455                2460
Leu Pro Thr Ser Leu Pro Ser Ser Leu Val Pro Pro Val Thr Ala Ala
2465                2470                2475                2480
Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro Val Asp
                2485                2490                2495
Asn Thr Pro Ser His Gln Leu Gln Val Pro Glu His Pro Phe Leu Thr
            2500                2505                2510
Pro Ser Pro Glu Ser Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser
        2515                2520                2525
Asn Val Ser Asp Trp Ser Glu Gly Val Ser Ser Pro Pro Thr Ser Met
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gtc tcc tct tgg ctc tgc cag gag ccg gag ccc tgc cac cct ggc ttt    96
Val Ser Ser Trp Leu Cys Gln Glu Pro Glu Pro Cys His Pro Gly Phe
            20                  25                  30

gac gcc gag agc tac acg ttc acg gtg ccc cgg cgc cac ctg gag aga    144
Asp Ala Glu Ser Tyr Thr Phe Thr Val Pro Arg Arg His Leu Glu Arg
        35                  40                  45

ggc cgc gtc ctg ggc aga gtg aat ttt gaa gat tgc acc ggt cga caa    192
Gly Arg Val Leu Gly Arg Val Asn Phe Glu Asp Cys Thr Gly Arg Gln
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Arg Thr Ala Tyr Phe Ser Leu Asp Thr Arg Phe Lys Val Gly Thr Asp
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ggt gtg att aca gtc aaa agg cct cta cgg ttt cat aac cca cag atc      288
Gly Val Ile Thr Val Lys Arg Pro Leu Arg Phe His Asn Pro Gln Ile
                 85                  90                  95

cat ttc ttg gtc tac gcc tgg gac tcc acc tac aga aag ttt tcc acc      336
His Phe Leu Val Tyr Ala Trp Asp Ser Thr Tyr Arg Lys Phe Ser Thr
            100                 105                 110

aaa gtc acg ctg aat aca gtg ggg cac cac cac cgc ccc ccg ccc cat      384
Lys Val Thr Leu Asn Thr Val Gly His His His Arg Pro Pro Pro His
        115                 120                 125

cag gcc tcc gtt tct gga atc caa gca gaa ttg ctc aca ttt ccc aac      432
Gln Ala Ser Val Ser Gly Ile Gln Ala Glu Leu Leu Thr Phe Pro Asn
    130                 135                 140

tcc tct cct ggc ctc aga aga cag aag aga gac tgg gtt att cct ccc      480
Ser Ser Pro Gly Leu Arg Arg Gln Lys Arg Asp Trp Val Ile Pro Pro
145                 150                 155                 160

atc agc tgc cca gaa aat gaa aaa ggc cca ttt cct aaa aac ctg gtt      528
Ile Ser Cys Pro Glu Asn Glu Lys Gly Pro Phe Pro Lys Asn Leu Val
                165                 170                 175

cag atc aaa tcc aac aaa gac aaa gaa ggc aag gtt ttc tac agc atc      576
Gln Ile Lys Ser Asn Lys Asp Lys Glu Gly Lys Val Phe Tyr Ser Ile
            180                 185                 190

act ggc caa gga gct gac aca ccc cct gtt ggt gtc ttt att att gaa      624
Thr Gly Gln Gly Ala Asp Thr Pro Pro Val Gly Val Phe Ile Ile Glu
        195                 200                 205

aga gaa aca gga tgg ctg aag gtg aca gag cct ctg gat aga gaa cgc      672
Arg Glu Thr Gly Trp Leu Lys Val Thr Glu Pro Leu Asp Arg Glu Arg
    210                 215                 220

att gcc aca tac act ctc ttc tct cac gct gtg tca tcc aac ggg aat      720
Ile Ala Thr Tyr Thr Leu Phe Ser His Ala Val Ser Ser Asn Gly Asn
225                 230                 235                 240

gca gtt gag gat cca atg gag att ttg atc acg gta acc gat cag aat      768
Ala Val Glu Asp Pro Met Glu Ile Leu Ile Thr Val Thr Asp Gln Asn
                245                 250                 255

gac aac aag ccc gaa ttc acc cag gag gtc ttt aag ggg tct gtc atg      816
Asp Asn Lys Pro Glu Phe Thr Gln Glu Val Phe Lys Gly Ser Val Met
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260 265 270

gaa ggt gct ctt cca gga acc tct gtg atg gag gtc aca gcc aca gac 864
Glu Gly Ala Leu Pro Gly Thr Ser Val Met Glu Val Thr Ala Thr Asp
275 280 285

gcg gac gat gat gtg aac acc tac aat gcc gcc atc gct tac acc atc 912
Ala Asp Asp Asp Val Asn Thr Tyr Asn Ala Ala Ile Ala Tyr Thr Ile
290 295 300

ctc agc caa gat cct gag ctc cct gac aaa aat atg ttc acc att aac 960
Leu Ser Gln Asp Pro Glu Leu Pro Asp Lys Asn Met Phe Thr Ile Asn
305 310 315 320

agg aac aca gga gtc atc agt gtg gtc acc act ggg ctg gac cga gag 1008
Arg Asn Thr Gly Val Ile Ser Val Val Thr Thr Gly Leu Asp Arg Glu
325 330 335

agt ttc cct acg tat acc ctg gtg gtt caa gct gct gac ctt caa ggt 1056
Ser Phe Pro Thr Tyr Thr Leu Val Val Gln Ala Ala Asp Leu Gln Gly
340 345 350

gag ggg tta agc aca aca gca aca gct gtg atc aca gtc act gac acc 1104
Glu Gly Leu Ser Thr Thr Ala Thr Ala Val Ile Thr Val Thr Asp Thr
355 360 365

aac gat aat cct ccg atc ttc aat ccc acc acg tac aag ggt cag gtg 1152
Asn Asp Asn Pro Pro Ile Phe Asn Pro Thr Thr Tyr Lys Gly Gln Val
370 375 380

cct gag aac gag gct aac gtc gta atc acc aca ctg aaa gtg act gat 1200
Pro Glu Asn Glu Ala Asn Val Val Ile Thr Thr Leu Lys Val Thr Asp
385 390 395 400

gct gat gcc ccc aat acc cca gcg tgg gag gct gta tac acc ata ttg 1248
Ala Asp Ala Pro Asn Thr Pro Ala Trp Glu Ala Val Tyr Thr Ile Leu
405 410 415

aat gat gat ggt gga caa ttt gtc gtc acc aca aat cca gtg aac aac 1296
Asn Asp Asp Gly Gly Gln Phe Val Val Thr Thr Asn Pro Val Asn Asn
420 425 430

gat ggc att ttg aaa aca gca aag ggc ttg gat ttt gag gcc aag cag 1344
Asp Gly Ile Leu Lys Thr Ala Lys Gly Leu Asp Phe Glu Ala Lys Gln
435 440 445

cag tac att cta cac gta gca gtg acg aat gtg gta cct ttt gag gtc 1392
Gln Tyr Ile Leu His Val Ala Val Thr Asn Val Val Pro Phe Glu Val
450 455 460

tct ctc acc acc tcc aca gcc acc gtc acc gtg gat gtg ctg gat gtg 1440
Ser Leu Thr Thr Ser Thr Ala Thr Val Thr Val Asp Val Leu Asp Val

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465                 470                 475                 480

aat gaa gcc ccc atc ttt gtg cct cct gaa aag aga gtg gaa gtg tcc      1488
Asn Glu Ala Pro Ile Phe Val Pro Pro Glu Lys Arg Val Glu Val Ser
                485                 490                 495

gag gac ttt ggc gtg ggc cag gaa atc aca tcc tac act gcc cag gag      1536
Glu Asp Phe Gly Val Gly Gln Glu Ile Thr Ser Tyr Thr Ala Gln Glu
            500                 505                 510

cca gac aca ttt atg gaa cag aaa ata aca tat cgg att tgg aga gac      1584
Pro Asp Thr Phe Met Glu Gln Lys Ile Thr Tyr Arg Ile Trp Arg Asp
        515                 520                 525

act gcc aac tgg ctg gag att aat ccg gac act ggt gcc att tcc act      1632
Thr Ala Asn Trp Leu Glu Ile Asn Pro Asp Thr Gly Ala Ile Ser Thr
    530                 535                 540

cgg gct gag ctg gac agg gag gat ttt gag cac gtg aag aac agc acg      1680
Arg Ala Glu Leu Asp Arg Glu Asp Phe Glu His Val Lys Asn Ser Thr
545                 550                 555                 560

tac aca gcc cta atc ata gct aca gac aat ggt tct cca gtt gct act      1728
Tyr Thr Ala Leu Ile Ile Ala Thr Asp Asn Gly Ser Pro Val Ala Thr
                565                 570                 575

gga aca ggg aca ctt ctg ctg atc ctg tct gat gtg aat gac aac gcc      1776
Gly Thr Gly Thr Leu Leu Leu Ile Leu Ser Asp Val Asn Asp Asn Ala
            580                 585                 590

ccc ata cca gaa cct cga act ata ttc ttc tgt gag agg aat cca aag      1824
Pro Ile Pro Glu Pro Arg Thr Ile Phe Phe Cys Glu Arg Asn Pro Lys
        595                 600                 605

cct cag gtc ata aac atc att gat gca gac ctt cct ccc aat aca tct      1872
Pro Gln Val Ile Asn Ile Ile Asp Ala Asp Leu Pro Pro Asn Thr Ser
    610                 615                 620

ccc ttc aca gca gaa cta aca cac ggg gcg agt gcc aac tgg acc att      1920
Pro Phe Thr Ala Glu Leu Thr His Gly Ala Ser Ala Asn Trp Thr Ile
625                 630                 635                 640

cag tac aac gac cca acc caa gaa tct atc att ttg aag cca aag atg      1968
Gln Tyr Asn Asp Pro Thr Gln Glu Ser Ile Ile Leu Lys Pro Lys Met
                645                 650                 655

gcc tta gag gtg ggt gac tac aaa atc aat ctc aag ctc atg gat aac      2016
Ala Leu Glu Val Gly Asp Tyr Lys Ile Asn Leu Lys Leu Met Asp Asn
            660                 665                 670

cag aat aaa gac caa gtg acc acc tta gag gtc agc gtg tgt gac tgt      2064
Gln Asn Lys Asp Gln Val Thr Thr Leu Glu Val Ser Val Cys Asp Cys
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        675                 680                 685

gaa ggg gcc gcc ggc gtc tgt agg aag gca cag cct gtc gaa gca gga    2112
Glu Gly Ala Ala Gly Val Cys Arg Lys Ala Gln Pro Val Glu Ala Gly
    690                 695                 700

ttg caa att cct gcc att ctg ggg att ctt gga gga att ctt gct ttg    2160
Leu Gln Ile Pro Ala Ile Leu Gly Ile Leu Gly Gly Ile Leu Ala Leu
705                 710                 715                 720

cta att ctg att ctg ctg ctc ttg ctg ttt ctt cgg agg aga gcg gtg    2208
Leu Ile Leu Ile Leu Leu Leu Leu Leu Phe Leu Arg Arg Arg Ala Val
                725                 730                 735

gtc aaa gag ccc tta ctg ccc cca gag gat gac acc cgg gac aac gtt    2256
Val Lys Glu Pro Leu Leu Pro Pro Glu Asp Asp Thr Arg Asp Asn Val
            740                 745                 750

tat tac tat gat gaa gaa gga ggc gga gaa gag gac cag gac ttt gac    2304
Tyr Tyr Tyr Asp Glu Glu Gly Gly Gly Glu Glu Asp Gln Asp Phe Asp
        755                 760                 765

ttg agc cag ctg cac agg ggc ctg gac gct cgg cct gaa gtg act cgt    2352
Leu Ser Gln Leu His Arg Gly Leu Asp Ala Arg Pro Glu Val Thr Arg
    770                 775                 780

aac gac gtt gca cca acc ctc atg agt gtc ccc cgg tat ctt ccc cgc    2400
Asn Asp Val Ala Pro Thr Leu Met Ser Val Pro Arg Tyr Leu Pro Arg
785                 790                 795                 800

cct gcc aat ccc gat gaa att gga aat ttt att gat gaa aat ctg aaa    2448
Pro Ala Asn Pro Asp Glu Ile Gly Asn Phe Ile Asp Glu Asn Leu Lys
                805                 810                 815

gcg gct gat act gac ccc aca gcc ccg cct tat gat tct ctg ctc gtg    2496
Ala Ala Asp Thr Asp Pro Thr Ala Pro Pro Tyr Asp Ser Leu Leu Val
            820                 825                 830

ttt gac tat gaa gga agc ggt tcc gaa gct gct agt ctg agc tcc ctg    2544
Phe Asp Tyr Glu Gly Ser Gly Ser Glu Ala Ala Ser Leu Ser Ser Leu
        835                 840                 845

aac tcc tca gag tca gac aaa gac cag gac tat gac tac ttg aac gaa    2592
Asn Ser Ser Glu Ser Asp Lys Asp Gln Asp Tyr Asp Tyr Leu Asn Glu
    850                 855                 860

tgg ggc aat cgc ttc aag aag ctg gct gac atg tac gga ggc ggc gag    2640
Trp Gly Asn Arg Phe Lys Lys Leu Ala Asp Met Tyr Gly Gly Gly Glu
865                 870                 875                 880

gac gac tag                                                        2649
Asp Asp
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            20                  25                  30
Asp Ala Glu Ser Tyr Thr Phe Thr Val Pro Arg Arg His Leu Glu Arg
        35                  40                  45
Gly Arg Val Leu Gly Arg Val Asn Phe Glu Asp Cys Thr Gly Arg Gln
    50                  55                  60
Arg Thr Ala Tyr Phe Ser Leu Asp Thr Arg Phe Lys Val Gly Thr Asp
65                  70                  75                  80
Gly Val Ile Thr Val Lys Arg Pro Leu Arg Phe His Asn Pro Gln Ile
                85                  90                  95
His Phe Leu Val Tyr Ala Trp Asp Ser Thr Tyr Arg Lys Phe Ser Thr
            100                 105                 110
Lys Val Thr Leu Asn Thr Val Gly His His His Arg Pro Pro Pro His
        115                 120                 125
Gln Ala Ser Val Ser Gly Ile Gln Ala Glu Leu Leu Thr Phe Pro Asn
    130                 135                 140
Ser Ser Pro Gly Leu Arg Arg Gln Lys Arg Asp Trp Val Ile Pro Pro
145                 150                 155                 160
Ile Ser Cys Pro Glu Asn Glu Lys Gly Pro Phe Pro Lys Asn Leu Val
                165                 170                 175
Gln Ile Lys Ser Asn Lys Asp Lys Glu Gly Lys Val Phe Tyr Ser Ile
            180                 185                 190
Thr Gly Gln Gly Ala Asp Thr Pro Pro Val Gly Val Phe Ile Ile Glu
        195                 200                 205
Arg Glu Thr Gly Trp Leu Lys Val Thr Glu Pro Leu Asp Arg Glu Arg
    210                 215                 220
Ile Ala Thr Tyr Thr Leu Phe Ser His Ala Val Ser Ser Asn Gly Asn
225                 230                 235                 240
Ala Val Glu Asp Pro Met Glu Ile Leu Ile Thr Val Thr Asp Gln Asn
                245                 250                 255
Asp Asn Lys Pro Glu Phe Thr Gln Glu Val Phe Lys Gly Ser Val Met
            260                 265                 270
Glu Gly Ala Leu Pro Gly Thr Ser Val Met Glu Val Thr Ala Thr Asp
        275                 280                 285
Ala Asp Asp Asp Val Asn Thr Tyr Asn Ala Ala Ile Ala Tyr Thr Ile
    290                 295                 300
Leu Ser Gln Asp Pro Glu Leu Pro Asp Lys Asn Met Phe Thr Ile Asn
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305                 310                 315                 320
Arg Asn Thr Gly Val Ile Ser Val Val Thr Thr Gly Leu Asp Arg Glu
                325                 330                 335
Ser Phe Pro Thr Tyr Thr Leu Val Val Gln Ala Ala Asp Leu Gln Gly
            340                 345                 350
Glu Gly Leu Ser Thr Thr Ala Thr Ala Val Ile Thr Val Thr Asp Thr
        355                 360                 365
Asn Asp Asn Pro Pro Ile Phe Asn Pro Thr Thr Tyr Lys Gly Gln Val
    370                 375                 380
Pro Glu Asn Glu Ala Asn Val Val Ile Thr Thr Leu Lys Val Thr Asp
385                 390                 395                 400
Ala Asp Ala Pro Asn Thr Pro Ala Trp Glu Ala Val Tyr Thr Ile Leu
                405                 410                 415
Asn Asp Asp Gly Gly Gln Phe Val Val Thr Thr Asn Pro Val Asn Asn
            420                 425                 430
Asp Gly Ile Leu Lys Thr Ala Lys Gly Leu Asp Phe Glu Ala Lys Gln
        435                 440                 445
Gln Tyr Ile Leu His Val Ala Val Thr Asn Val Val Pro Phe Glu Val
    450                 455                 460
Ser Leu Thr Thr Ser Thr Ala Thr Val Thr Val Asp Val Leu Asp Val
465                 470                 475                 480
Asn Glu Ala Pro Ile Phe Val Pro Pro Glu Lys Arg Val Glu Val Ser
                485                 490                 495
Glu Asp Phe Gly Val Gly Gln Glu Ile Thr Ser Tyr Thr Ala Gln Glu
            500                 505                 510
Pro Asp Thr Phe Met Glu Gln Lys Ile Thr Tyr Arg Ile Trp Arg Asp
        515                 520                 525
Thr Ala Asn Trp Leu Glu Ile Asn Pro Asp Thr Gly Ala Ile Ser Thr
    530                 535                 540
Arg Ala Glu Leu Asp Arg Glu Asp Phe Glu His Val Lys Asn Ser Thr
545                 550                 555                 560
Tyr Thr Ala Leu Ile Ile Ala Thr Asp Asn Gly Ser Pro Val Ala Thr
                565                 570                 575
Gly Thr Gly Thr Leu Leu Leu Ile Leu Ser Asp Val Asn Asp Asn Ala
            580                 585                 590
Pro Ile Pro Glu Pro Arg Thr Ile Phe Phe Cys Glu Arg Asn Pro Lys
        595                 600                 605
Pro Gln Val Ile Asn Ile Ile Asp Ala Asp Leu Pro Pro Asn Thr Ser
    610                 615                 620
Pro Phe Thr Ala Glu Leu Thr His Gly Ala Ser Ala Asn Trp Thr Ile
625                 630                 635                 640
Gln Tyr Asn Asp Pro Thr Gln Glu Ser Ile Ile Leu Lys Pro Lys Met
                645                 650                 655
Ala Leu Glu Val Gly Asp Tyr Lys Ile Asn Leu Lys Leu Met Asp Asn
            660                 665                 670
Gln Asn Lys Asp Gln Val Thr Thr Leu Glu Val Ser Val Cys Asp Cys
        675                 680                 685
Glu Gly Ala Ala Gly Val Cys Arg Lys Ala Gln Pro Val Glu Ala Gly
    690                 695                 700
Leu Gln Ile Pro Ala Ile Leu Gly Ile Leu Gly Gly Ile Leu Ala Leu
705                 710                 715                 720
Leu Ile Leu Ile Leu Leu Leu Leu Leu Phe Leu Arg Arg Arg Ala Val
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                725                 730                 735
Val Lys Glu Pro Leu Leu Pro Pro Glu Asp Asp Thr Arg Asp Asn Val
            740                 745                 750
Tyr Tyr Tyr Asp Glu Glu Gly Gly Gly Glu Glu Asp Gln Asp Phe Asp
        755                 760                 765
Leu Ser Gln Leu His Arg Gly Leu Asp Ala Arg Pro Glu Val Thr Arg
    770                 775                 780
Asn Asp Val Ala Pro Thr Leu Met Ser Val Pro Arg Tyr Leu Pro Arg
785                 790                 795                 800
Pro Ala Asn Pro Asp Glu Ile Gly Asn Phe Ile Asp Glu Asn Leu Lys
            805                 810                 815
Ala Ala Asp Thr Asp Pro Thr Ala Pro Pro Tyr Asp Ser Leu Leu Val
            820                 825                 830
Phe Asp Tyr Glu Gly Ser Gly Ser Glu Ala Ala Ser Leu Ser Ser Leu
        835                 840                 845
Asn Ser Ser Glu Ser Asp Lys Asp Gln Asp Tyr Asp Tyr Leu Asn Glu
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Trp Gly Asn Arg Phe Lys Lys Leu Ala Asp Met Tyr Gly Gly Gly Glu
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Asp Asp
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Trp Val Trp Val Ser Leu Leu Val Ala Ala Gly Thr Val Gln Pro Ser
        10                  15                  20

gat tct cag tca gtg tgt gca gga acg gag aat aaa ctg agc tct ctc   150
Asp Ser Gln Ser Val Cys Ala Gly Thr Glu Asn Lys Leu Ser Ser Leu
    25                  30                  35

tct gac ctg gaa cag cag tac cga gcc ttg cgc aag tac tat gaa aac   198
Ser Asp Leu Glu Gln Gln Tyr Arg Ala Leu Arg Lys Tyr Tyr Glu Asn
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40                  45                  50                  55

tgt gag gtt gtc atg ggc aac ctg gag ata acc agc att gag cac aac     246
Cys Glu Val Val Met Gly Asn Leu Glu Ile Thr Ser Ile Glu His Asn
                60                  65                  70

cgg gac ctc tcc ttc ctg cgg tct gtt cga gaa gtc aca ggc tac gtg     294
Arg Asp Leu Ser Phe Leu Arg Ser Val Arg Glu Val Thr Gly Tyr Val
            75                  80                  85

tta gtg gct ctt aat cag ttt cgt tac ctg cct ctg gag aat tta cgc     342
Leu Val Ala Leu Asn Gln Phe Arg Tyr Leu Pro Leu Glu Asn Leu Arg
        90                  95                  100

att att cgt ggg aca aaa ctt tat gag gat cga tat gcc ttg gca ata     390
Ile Ile Arg Gly Thr Lys Leu Tyr Glu Asp Arg Tyr Ala Leu Ala Ile
    105                 110                 115

ttt tta aac tac aga aaa gat gga aac ttt gga ctt caa gaa ctt gga     438
Phe Leu Asn Tyr Arg Lys Asp Gly Asn Phe Gly Leu Gln Glu Leu Gly
120                 125                 130                 135

tta aag aac ttg aca gaa atc cta aat ggt gga gtc tat gta gac cag     486
Leu Lys Asn Leu Thr Glu Ile Leu Asn Gly Gly Val Tyr Val Asp Gln
                140                 145                 150

aac aaa ttc ctt tgt tat gca gac acc att cat tgg caa gat att gtt     534
Asn Lys Phe Leu Cys Tyr Ala Asp Thr Ile His Trp Gln Asp Ile Val
            155                 160                 165

cgg aac cca tgg cct tcc aac ttg act ctt gtg tca aca aat ggt agt     582
Arg Asn Pro Trp Pro Ser Asn Leu Thr Leu Val Ser Thr Asn Gly Ser
        170                 175                 180

tca gga tgt gga cgt tgc cat aag tcc tgt act ggc cgt tgc tgg gga     630
Ser Gly Cys Gly Arg Cys His Lys Ser Cys Thr Gly Arg Cys Trp Gly
    185                 190                 195

ccc aca gaa aat cat tgc cag act ttg aca agg acg gtg tgt gca gaa     678
Pro Thr Glu Asn His Cys Gln Thr Leu Thr Arg Thr Val Cys Ala Glu
200                 205                 210                 215

caa tgt gac ggc aga tgc tac gga cct tac gtc agt gac tgc tgc cat     726
Gln Cys Asp Gly Arg Cys Tyr Gly Pro Tyr Val Ser Asp Cys Cys His
                220                 225                 230

cga gaa tgt gct gga ggc tgc tca gga cct aag gac aca gac tgc ttt     774
Arg Glu Cys Ala Gly Gly Cys Ser Gly Pro Lys Asp Thr Asp Cys Phe
            235                 240                 245

gcc tgc atg aat ttc aat gac agt gga gca tgt gtt act cag tgt ccc     822
Ala Cys Met Asn Phe Asn Asp Ser Gly Ala Cys Val Thr Gln Cys Pro
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250 255 260

caa acc ttt gtc tac aat cca acc acc ttt caa ctg gag cac aat ttc 870
Gln Thr Phe Val Tyr Asn Pro Thr Thr Phe Gln Leu Glu His Asn Phe
265 270 275

aat gca aag tac aca tat gga gca ttc tgt gtc aag aaa tgt cca cat 918
Asn Ala Lys Tyr Thr Tyr Gly Ala Phe Cys Val Lys Lys Cys Pro His
280 285 290 295

aac ttt gtg gta gat tcc agt tct tgt gtg cgt gcc tgc cct agt tcc 966
Asn Phe Val Val Asp Ser Ser Ser Cys Val Arg Ala Cys Pro Ser Ser
300 305 310

aag atg gaa gta gaa gaa aat ggg att aaa atg tgt aaa cct tgc act 1014
Lys Met Glu Val Glu Glu Asn Gly Ile Lys Met Cys Lys Pro Cys Thr
315 320 325

gac att tgc cca aaa gct tgt gat ggc att ggc aca gga tca ttg atg 1062
Asp Ile Cys Pro Lys Ala Cys Asp Gly Ile Gly Thr Gly Ser Leu Met
330 335 340

tca gct cag act gtg gat tcc agt aac att gac aaa ttc ata aac tgt 1110
Ser Ala Gln Thr Val Asp Ser Ser Asn Ile Asp Lys Phe Ile Asn Cys
345 350 355

acc aag atc aat ggg aat ttg atc ttt cta gtc act ggt att cat ggg 1158
Thr Lys Ile Asn Gly Asn Leu Ile Phe Leu Val Thr Gly Ile His Gly
360 365 370 375

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Pro Pro Asn Met Thr Asp Phe Ser Val Phe Ser Asn Leu Val Thr Ile
410 415 420

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Gly Gly Arg Val Leu Tyr Ser Gly Leu Ser Leu Leu Ile Leu Lys Gln
425 430 435

cag ggc atc acc tct cta cag ttc cag tcc ctg aag gaa atc agc gca 1398
Gln Gly Ile Thr Ser Leu Gln Phe Gln Ser Leu Lys Glu Ile Ser Ala
440 445 450 455

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Gly Asn Ile Tyr Ile Thr Asp Asn Ser Asn Leu Cys Tyr Tyr His Thr

460 465 470

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Ile Asn Trp Thr Thr Leu Phe Ser Thr Ile Asn Gln Arg Ile Val Ile
475 480 485

cgg gac aac aga aaa gct gaa aat tgt act gct gaa gga atg gtg tgc 1542
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490 495 500

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Asn His Leu Cys Ser Ser Asp Gly Cys Trp Gly Pro Gly Pro Asp Gln
505 510 515

tgt ctg tcg tgt cgc cgc ttc agt aga gga agg atc tgc ata gag tct 1638
Cys Leu Ser Cys Arg Arg Phe Ser Arg Gly Arg Ile Cys Ile Glu Ser
520 525 530 535

tgt aac ctc tat gat ggt gaa ttt cgg gag ttt gag aat ggc tcc atc 1686
Cys Asn Leu Tyr Asp Gly Glu Phe Arg Glu Phe Glu Asn Gly Ser Ile
540 545 550

tgt gtg gag tgt gac ccc cag tgt gag aag atg gaa gat ggc ctc ctc 1734
Cys Val Glu Cys Asp Pro Gln Cys Glu Lys Met Glu Asp Gly Leu Leu
555 560 565

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570 575 580

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Lys Asp Gly Pro Asn Cys Val Glu Lys Cys Pro Asp Gly Leu Gln Gly
585 590 595

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Pro Cys His Pro Asn Cys Thr Gln Gly Cys Asn Gly Pro Thr Ser His
620 625 630

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Asp Cys Ile Tyr Tyr Pro Trp Thr Gly His Ser Thr Leu Pro Gln His
635 640 645

gct aga act ccc ctg att gca gct gga gta att ggt ggg ctc ttc att 2022
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650 655 660

ctg gtc att gtg ggt ctg aca ttt gct gtt tat gtt aga agg aag agc 2070
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665 670 675

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715 720 725

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730 735 740

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Val Lys Ile Pro Val Ala Ile Lys Ile Leu Asn Glu Thr Thr Gly Pro
745 750 755

aag gca aat gtg gag ttc atg gat gaa gct ctg atc atg gca agt atg 2358
Lys Ala Asn Val Glu Phe Met Asp Glu Ala Leu Ile Met Ala Ser Met
760 765 770 775

gat cat cca cac cta gtc cgg ttg ctg ggt gtg tgt ctg agc cca acc 2406
Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser Pro Thr
780 785 790

atc cag ctg gtt act caa ctt atg ccc cat ggc tgc ctg ttg gag tat 2454
Ile Gln Leu Val Thr Gln Leu Met Pro His Gly Cys Leu Leu Glu Tyr
795 800 805

gtc cac gag cac aag gat aac att gga tca caa ctg ctg ctt aac tgg 2502
Val His Glu His Lys Asp Asn Ile Gly Ser Gln Leu Leu Leu Asn Trp
810 815 820

tgt gtc cag ata gct aag gga atg atg tac ctg gaa gaa aga cga ctc 2550
Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Glu Arg Arg Leu
825 830 835

gtt cat cgg gat ttg gca gcc cgt aat gtc tta gtg aaa tct cca aac 2598
Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Ser Pro Asn
840 845 850 855

cat gtg aaa atc aca gat ttt ggg cta gcc aga ctc ttg gaa gga gat 2646
His Val Lys Ile Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu Gly Asp
860 865 870

gaa aaa gag tac aat gct gat gga gga aag atg cca att aaa tgg atg 2694
Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys Trp Met

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            875                 880                 885

gct ctg gag tgt ata cat tac agg aaa ttc acc cat cag agt gac gtt     2742
Ala Leu Glu Cys Ile His Tyr Arg Lys Phe Thr His Gln Ser Asp Val
        890                 895                 900

tgg agc tat gga gtt act ata tgg gaa ctg atg acc ttt gga gga aaa     2790
Trp Ser Tyr Gly Val Thr Ile Trp Glu Leu Met Thr Phe Gly Gly Lys
    905                 910                 915

ccc tat gat gga att cca acg cga gaa atc cct gat tta tta gag aaa     2838
Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu Glu Lys
920                 925                 930                 935

gga gaa cgt ttg cct cag cct ccc atc tgc act att gac gtt tac atg     2886
Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr Met
                940                 945                 950

gtc atg gtc aaa tgt tgg atg att gat gct gac agt aga cct aaa ttt     2934
Val Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys Phe
            955                 960                 965

aag gaa ctg gct gct gag ttt tca agg atg gct cga gac cct caa aga     2982
Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg Asp Pro Gln Arg
        970                 975                 980

tac cta gtt att cag ggt gat gat cgt atg aag ctt ccc agt cca aat     3030
Tyr Leu Val Ile Gln Gly Asp Asp Arg Met Lys Leu Pro Ser Pro Asn
    985                 990                 995

gac agc aag ttc ttt cag aat ctc ttg gat gaa gag gat ttg gaa gat     3078
Asp Ser Lys Phe Phe Gln Asn Leu Leu Asp Glu Glu Asp Leu Glu Asp
1000                1005                1010                1015

atg atg gat gct gag gag tac ttg gtc cct cag gct ttc aac atc cca     3126
Met Met Asp Ala Glu Glu Tyr Leu Val Pro Gln Ala Phe Asn Ile Pro
                1020                1025                1030

cct ccc atc tat act tcc aga gca aga att gac tcg aat agg agt gaa     3174
Pro Pro Ile Tyr Thr Ser Arg Ala Arg Ile Asp Ser Asn Arg Ser Glu
            1035                1040                1045

att gga cac agc cct cct cct gcc tac acc ccc atg tca gga aac cag     3222
Ile Gly His Ser Pro Pro Pro Ala Tyr Thr Pro Met Ser Gly Asn Gln
        1050                1055                1060

ttt gta tac cga gat gga ggt ttt gct gct gaa caa gga gtg tct gtg     3270
Phe Val Tyr Arg Asp Gly Gly Phe Ala Ala Glu Gln Gly Val Ser Val
    1065                1070                1075

ccc tac aga gcc cca act agc aca att cca gaa gct cct gtg gca cag     3318
Pro Tyr Arg Ala Pro Thr Ser Thr Ile Pro Glu Ala Pro Val Ala Gln
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1080                1085                1090                1095

ggt gct act gct gag att ttt gat gac tcc tgc tgt aat ggc acc cta    3366
Gly Ala Thr Ala Glu Ile Phe Asp Asp Ser Cys Cys Asn Gly Thr Leu
                1100                1105                1110

cgc aag cca gtg gca ccc cat gtc caa gag gac agt agc acc cag agg    3414
Arg Lys Pro Val Ala Pro His Val Gln Glu Asp Ser Ser Thr Gln Arg
            1115                1120                1125

tac agt gct gac ccc acc gtg ttt gcc cca gaa cgg agc cca cga gga    3462
Tyr Ser Ala Asp Pro Thr Val Phe Ala Pro Glu Arg Ser Pro Arg Gly
        1130                1135                1140

gag ctg gat gag gaa ggt tac atg act cct atg cga gac aaa ccc aaa    3510
Glu Leu Asp Glu Glu Gly Tyr Met Thr Pro Met Arg Asp Lys Pro Lys
    1145                1150                1155

caa gaa tac ctg aat cca gtg gag gag aac cct ttt gtt tct cgg aga    3558
Gln Glu Tyr Leu Asn Pro Val Glu Glu Asn Pro Phe Val Ser Arg Arg
1160                1165                1170                1175

aaa aat gga gac ctt caa gca ttg gat aat ccc gaa tat cac aat gca    3606
Lys Asn Gly Asp Leu Gln Ala Leu Asp Asn Pro Glu Tyr His Asn Ala
                1180                1185                1190

tcc aat ggt cca ccc aag gcc gag gat gag tat gtg aat gag cca ctg    3654
Ser Asn Gly Pro Pro Lys Ala Glu Asp Glu Tyr Val Asn Glu Pro Leu
            1195                1200                1205

tac ctc aac acc ttt gcc aac acc ttg gga aaa gct gag tac ctg aag    3702
Tyr Leu Asn Thr Phe Ala Asn Thr Leu Gly Lys Ala Glu Tyr Leu Lys
        1210                1215                1220

aac aac ata ctg tca atg cca gag aag gcc aag aaa gcg ttt gac aac    3750
Asn Asn Ile Leu Ser Met Pro Glu Lys Ala Lys Lys Ala Phe Asp Asn
    1225                1230                1235

cct gac tac tgg aac cac agc ctg cca cct cgg agc acc ctt cag cac    3798
Pro Asp Tyr Trp Asn His Ser Leu Pro Pro Arg Ser Thr Leu Gln His
1240                1245                1250                1255

cca gac tac ctg cag gag tac agc aca aaa tat ttt tat aaa cag aat    3846
Pro Asp Tyr Leu Gln Glu Tyr Ser Thr Lys Tyr Phe Tyr Lys Gln Asn
                1260                1265                1270

ggg cgg atc cgg cct att gtg gca gag aat cct gaa tac ctc tct gag    3894
Gly Arg Ile Arg Pro Ile Val Ala Glu Asn Pro Glu Tyr Leu Ser Glu
            1275                1280                1285

ttc tcc ctg aag cca ggc act gtg ctg ccg cct cca cct tac aga cac    3942
Phe Ser Leu Lys Pro Gly Thr Val Leu Pro Pro Pro Pro Tyr Arg His
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1290 1295 1300

cgg aat act gtg gtg taa gctcagttgt ggttttttag gtggagagac 3990
Arg Asn Thr Val Val
1305

acacctgctc caatttcccc accccccctct ctttctctgg tggtcttcct tctaccccaa 4050
ggccagtagt tttgacactt cccagtggaa gatacagaga tgcaatgata gttatgtgct 4110
tacctaactt gaacattaga gggaaagact gaaagagaaa gataggagga accacaatgt 4170
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ttctttgttt ctttcttcct cttctttttt tttttttttt taaagcagat ggttgaaaca 4350
cccatgctat ctgttcctat ctgcaggaac tgatgtgtgc atatttagca tccctggaaa 4410
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tgaaattgag aatccagttt ctttccccag cagtttctgt cctagcaagt aagaatggcc 4530
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tatttttggc tttaatttct aattgcaaag atgtttacat caaagcttct tcacagaatt 4710
taagcaagaa atattttaat atagtgaaat ggccactact ttaagtatac aatctttaaa 4770
ataagaaagg gaggctaata tttttcatgc tatcaaatta tcttcaccct catcctttac 4830
atttttcaac attttttttt ctccataaat gacactactt gataggccgt tggttgtctg 4890
aagagtagaa gggaaactaa gagacagttc tctgtggttc aggaaaacta ctgatacttt 4950
caggggtggc ccaatgaggg aatccattga actggaagaa acacactgga ttgggtatgt 5010
ctacctggca gatactcaga aatgtagttt gcacttaagc tgtaatttta tttgttcttt 5070
ttctgaactc cattttggat tttgaatcaa gcaatatgga agcaaccagc aaattaacta 5130
atttaagtac atttttaaaa aaagagctaa gataaagact gtggaaatgc caaaccaagc 5190
aaattaggaa ccttgcaacg gtatccaggg actatgatga gaggccagca cattatcttc 5250
atatgtcacc tttgctacgc aaggaaattt gttcagttcg tatacttcgt aagaaggaat 5310
gcgagtaagg attggcttga attccatgga atttctagta tgagactatt tatatgaagt 5370
agaaggtaac tctttgcaca taaattggta taataaaaag aaaaacacaa acattcaaag 5430
cttagggata ggtccttggg tcaaaagttg taaataaatg tgaaacatct tctc 5484

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<212> PRT
<213> Homo Sapiens

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<309> 1995-01-09

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Ala Gly Thr Val Gln Pro Ser Asp Ser Gln Ser Val Cys Ala Gly Thr
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Glu Asn Lys Leu Ser Ser Leu Ser Asp Leu Glu Gln Gln Tyr Arg Ala
35 40 45
Leu Arg Lys Tyr Tyr Glu Asn Cys Glu Val Val Met Gly Asn Leu Glu
50 55 60
Ile Thr Ser Ile Glu His Asn Arg Asp Leu Ser Phe Leu Arg Ser Val
65 70 75 80

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Arg Glu Val Thr Gly Tyr Val Leu Val Ala Leu Asn Gln Phe Arg Tyr
                85                  90                  95
Leu Pro Leu Glu Asn Leu Arg Ile Ile Arg Gly Thr Lys Leu Tyr Glu
            100                 105                 110
Asp Arg Tyr Ala Leu Ala Ile Phe Leu Asn Tyr Arg Lys Asp Gly Asn
        115                 120                 125
Phe Gly Leu Gln Glu Leu Gly Leu Lys Asn Leu Thr Glu Ile Leu Asn
    130                 135                 140
Gly Gly Val Tyr Val Asp Gln Asn Lys Phe Leu Cys Tyr Ala Asp Thr
145                 150                 155                 160
Ile His Trp Gln Asp Ile Val Arg Asn Pro Trp Pro Ser Asn Leu Thr
                165                 170                 175
Leu Val Ser Thr Asn Gly Ser Ser Gly Cys Gly Arg Cys His Lys Ser
            180                 185                 190
Cys Thr Gly Arg Cys Trp Gly Pro Thr Glu Asn His Cys Gln Thr Leu
        195                 200                 205
Thr Arg Thr Val Cys Ala Glu Gln Cys Asp Gly Arg Cys Tyr Gly Pro
    210                 215                 220
Tyr Val Ser Asp Cys Cys His Arg Glu Cys Ala Gly Gly Cys Ser Gly
225                 230                 235                 240
Pro Lys Asp Thr Asp Cys Phe Ala Cys Met Asn Phe Asn Asp Ser Gly
                245                 250                 255
Ala Cys Val Thr Gln Cys Pro Gln Thr Phe Val Tyr Asn Pro Thr Thr
            260                 265                 270
Phe Gln Leu Glu His Asn Phe Asn Ala Lys Tyr Thr Tyr Gly Ala Phe
        275                 280                 285
Cys Val Lys Lys Cys Pro His Asn Phe Val Val Asp Ser Ser Ser Cys
    290                 295                 300
Val Arg Ala Cys Pro Ser Ser Lys Met Glu Val Glu Glu Asn Gly Ile
305                 310                 315                 320
Lys Met Cys Lys Pro Cys Thr Asp Ile Cys Pro Lys Ala Cys Asp Gly
                325                 330                 335
Ile Gly Thr Gly Ser Leu Met Ser Ala Gln Thr Val Asp Ser Ser Asn
            340                 345                 350
Ile Asp Lys Phe Ile Asn Cys Thr Lys Ile Asn Gly Asn Leu Ile Phe
        355                 360                 365
Leu Val Thr Gly Ile His Gly Asp Pro Tyr Asn Ala Ile Glu Ala Ile
    370                 375                 380
Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly
385                 390                 395                 400
Phe Leu Asn Ile Gln Ser Trp Pro Pro Asn Met Thr Asp Phe Ser Val
                405                 410                 415
Phe Ser Asn Leu Val Thr Ile Gly Gly Arg Val Leu Tyr Ser Gly Leu
            420                 425                 430
Ser Leu Leu Ile Leu Lys Gln Gln Gly Ile Thr Ser Leu Gln Phe Gln
        435                 440                 445
Ser Leu Lys Glu Ile Ser Ala Gly Asn Ile Tyr Ile Thr Asp Asn Ser
    450                 455                 460
Asn Leu Cys Tyr Tyr His Thr Ile Asn Trp Thr Thr Leu Phe Ser Thr
465                 470                 475                 480
Ile Asn Gln Arg Ile Val Ile Arg Asp Asn Arg Lys Ala Glu Asn Cys
                485                 490                 495
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Thr Ala Glu Gly Met Val Cys Asn His Leu Cys Ser Ser Asp Gly Cys
500 505 510
Trp Gly Pro Gly Pro Asp Gln Cys Leu Ser Cys Arg Arg Phe Ser Arg
515 520 525
Gly Arg Ile Cys Ile Glu Ser Cys Asn Leu Tyr Asp Gly Glu Phe Arg
530 535 540
Glu Phe Glu Asn Gly Ser Ile Cys Val Glu Cys Asp Pro Gln Cys Glu
545 550 555 560
Lys Met Glu Asp Gly Leu Leu Thr Cys His Gly Pro Gly Pro Asp Asn
565 570 575
Cys Thr Lys Cys Ser His Phe Lys Asp Gly Pro Asn Cys Val Glu Lys
580 585 590
Cys Pro Asp Gly Leu Gln Gly Ala Asn Ser Phe Ile Phe Lys Tyr Ala
595 600 605
Asp Pro Asp Arg Glu Cys His Pro Cys His Pro Asn Cys Thr Gln Gly
610 615 620
Cys Asn Gly Pro Thr Ser His Asp Cys Ile Tyr Tyr Pro Trp Thr Gly
625 630 635 640
His Ser Thr Leu Pro Gln His Ala Arg Thr Pro Leu Ile Ala Ala Gly
645 650 655
Val Ile Gly Gly Leu Phe Ile Leu Val Ile Val Gly Leu Thr Phe Ala
660 665 670
Val Tyr Val Arg Arg Lys Ser Ile Lys Lys Lys Arg Ala Leu Arg Arg
675 680 685
Phe Leu Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Thr Ala
690 695 700
Pro Asn Gln Ala Gln Leu Arg Ile Leu Lys Glu Thr Glu Leu Lys Arg
705 710 715 720
Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Ile
725 730 735
Trp Val Pro Glu Gly Glu Thr Val Lys Ile Pro Val Ala Ile Lys Ile
740 745 750
Leu Asn Glu Thr Thr Gly Pro Lys Ala Asn Val Glu Phe Met Asp Glu
755 760 765
Ala Leu Ile Met Ala Ser Met Asp His Pro His Leu Val Arg Leu Leu
770 775 780
Gly Val Cys Leu Ser Pro Thr Ile Gln Leu Val Thr Gln Leu Met Pro
785 790 795 800
His Gly Cys Leu Leu Glu Tyr Val His Glu His Lys Asp Asn Ile Gly
805 810 815
Ser Gln Leu Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met Met
820 825 830
Tyr Leu Glu Glu Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn
835 840 845
Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe Gly Leu
850 855 860
Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly Gly
865 870 875 880
Lys Met Pro Ile Lys Trp Met Ala Leu Glu Cys Ile His Tyr Arg Lys
885 890 895
Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Ile Trp Glu
900 905 910

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Leu Met Thr Phe Gly Gly Lys Pro Tyr Asp Gly Ile Pro Thr Arg Glu
        915                 920                 925
Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile
    930                 935                 940
Cys Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp
945                 950                 955                 960
Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg
                965                 970                 975
Met Ala Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp Arg
            980                 985                 990
Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu Leu
        995                 1000                1005
Asp Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu Val
    1010                1015                1020
Pro Gln Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala Arg
1025                1030                1035                1040
Ile Asp Ser Asn Arg Ser Glu Ile Gly His Ser Pro Pro Pro Ala Tyr
                1045                1050                1055
Thr Pro Met Ser Gly Asn Gln Phe Val Tyr Arg Asp Gly Gly Phe Ala
            1060                1065                1070
Ala Glu Gln Gly Val Ser Val Pro Tyr Arg Ala Pro Thr Ser Thr Ile
        1075                1080                1085
Pro Glu Ala Pro Val Ala Gln Gly Ala Thr Ala Glu Ile Phe Asp Asp
    1090                1095                1100
Ser Cys Cys Asn Gly Thr Leu Arg Lys Pro Val Ala Pro His Val Gln
1105                1110                1115                1120
Glu Asp Ser Ser Thr Gln Arg Tyr Ser Ala Asp Pro Thr Val Phe Ala
                1125                1130                1135
Pro Glu Arg Ser Pro Arg Gly Glu Leu Asp Glu Glu Gly Tyr Met Thr
            1140                1145                1150
Pro Met Arg Asp Lys Pro Lys Gln Glu Tyr Leu Asn Pro Val Glu Glu
        1155                1160                1165
Asn Pro Phe Val Ser Arg Arg Lys Asn Gly Asp Leu Gln Ala Leu Asp
    1170                1175                1180
Asn Pro Glu Tyr His Asn Ala Ser Asn Gly Pro Pro Lys Ala Glu Asp
1185                1190                1195                1200
Glu Tyr Val Asn Glu Pro Leu Tyr Leu Asn Thr Phe Ala Asn Thr Leu
                1205                1210                1215
Gly Lys Ala Glu Tyr Leu Lys Asn Asn Ile Leu Ser Met Pro Glu Lys
            1220                1225                1230
Ala Lys Lys Ala Phe Asp Asn Pro Asp Tyr Trp Asn His Ser Leu Pro
        1235                1240                1245
Pro Arg Ser Thr Leu Gln His Pro Asp Tyr Leu Gln Glu Tyr Ser Thr
    1250                1255                1260
Lys Tyr Phe Tyr Lys Gln Asn Gly Arg Ile Arg Pro Ile Val Ala Glu
1265                1270                1275                1280
Asn Pro Glu Tyr Leu Ser Glu Phe Ser Leu Lys Pro Gly Thr Val Leu
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Pro Pro Pro Pro Tyr Arg His Arg Asn Thr Val Val
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<210> 37
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<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(478)
<223> partial nucleotide sequence encoding A387 light
(kappa) chain

<221> variation
<222> 15
<223> n is any

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48
Asp Xaa Xaa Xaa Thr Gln Xaa Xaa Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15

gat agc gtc agt ctt tcc tgc agg gcc agc caa agt atc agc aac aac
96
Asp Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
20 25 30

cta cac tgg tat caa caa aaa tca cat gag tct cca agg att ctc atc
144
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Ile Leu Ile
35 40 45

aag tat gca tcc cag tcc atc tat ggg atc ccc tca agg ttc agt ggc
192
Lys Tyr Ala Ser Gln Ser Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

agt gga tca ggg aca ttt ttc act ctc att gtc aac agt gtg ggg act
240
Ser Gly Ser Gly Thr Phe Phe Thr Leu Ile Val Asn Ser Val Gly Thr
65 70 75 80

gaa gat ttt gga atg tat ttc tgt caa cag agt cac agc tgg cct ctc
288
Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser His Ser Trp Pro Leu
85 90 95

acg ttc ggt act ggg acc aag ctg gag ctg aaa cgg gct gat gct gca
336
Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala
100 105 110

cca act gta tcc atc ttc cca cca tcc agt gag cag tta aca tct gga
384
Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115 120 125

ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc tac ccc aga gac atc
432
Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Arg Asp Ile
130 135 140

aat gtc aag tgg aag att gat ggc agt gaa cga caa aat ggc gtc c
478
Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val
145 150 155

<210> 38
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<221> VARIANT
<222> 3
<223> Xaa is Val

<221> VARIANT
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<223> Xaa is Leu

<221> VARIANT
<222> 5
<223> Xaa is Thr

<221> VARIANT
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<223> Xaa is Thr, Arg, Ser, Cys or Trp

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Asp Xaa Xaa Xaa Thr Gln Xaa Xaa Ala Thr Leu Ser Val Ser Pro Gly
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Asp Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
20 25 30
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Ile Leu Ile
35 40 45
Lys Tyr Ala Ser Gln Ser Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Phe Phe Thr Leu Ile Val Asn Ser Val Gly Thr

65 70 75 80
Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser His Ser Trp Pro Leu
85 90 95
Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala
100 105 110
Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115 120 125
Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Arg Asp Ile
130 135 140
Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val
145 150 155

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<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(366)
<223> partial nucleotide sequence encoding A387 heavy
(igG2a) chain sequence

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Glu Val Lys Xaa Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
1 5 10 15

tcc ctg aaa ctc gcc tgt gca gcc tct gga ttc act ttc agt aac gat
96
Ser Leu Lys Leu Ala Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Asp
20 25 30

gcc atg tct tgg gtt cgc cag act cca gaa aag agg ctg gag tgg gtc
144
Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

gca tcc att agt agt gtt ggt aac acc tac tat cca gac agt gtg aag
192
Ala Ser Ile Ser Ser Val Gly Asn Thr Tyr Tyr Pro Asp Ser Val Lys
50 55 60

ggc cga ttc acc atc tcc aga gat aat gcc agg aac att cta tac ctg
240
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
65 70 75 80

caa atg agt agt gtg agg tct gag gac acg gcc atg tat tac tgt gca
288
Gln Met Ser Ser Val Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala

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                  85                  90                  95

aga ggc tat ggt gtt agt ccc tgg ttt tct tac tgg ggc caa ggg act
336
Arg Gly Tyr Gly Val Ser Pro Trp Phe Ser Tyr Trp Gly Gln Gly Thr
            100                 105                 110

cta gtc acc gtc tcc tca gcc aaa aca aca
366
Leu Val Thr Val Ser Ser Ala Lys Thr Thr
        115                 120
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<223> Xaa is Leu or Phe

<400> 40

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            20                  25                  30
Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
        35                  40                  45
Ala Ser Ile Ser Ser Val Gly Asn Thr Tyr Tyr Pro Asp Ser Val Lys
    50                  55                  60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
65                  70                  75                  80
Gln Met Ser Ser Val Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala
                85                  90                  95
Arg Gly Tyr Gly Val Ser Pro Trp Phe Ser Tyr Trp Gly Gln Gly Thr
            100                 105                 110
Leu Val Thr Val Ser Ser Ala Lys Thr Thr
        115                 120
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<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(493)
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Light Chain V-J region

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1 5 10 15

gat caa gcc tcc atc tct tgc aga tct agt cag aac att gta cat agt
96
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser
20 25 30

agt gga aac acc tat tta gaa tgg tac ctg cag aaa cca ggc cag tct
144
Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca
192
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc
240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

agc aga gtg gag gct gag gat ctg gga att tat tac tgc ttt caa ggt
288
Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly
85 90 95

tca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa
336
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

cgg gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag
384
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
115 120 125

cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc
432
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
130 135 140

tac ccc aga gac atc aat gtc aag tgg aag att gat ggc agt gaa cga
480
Tyr Pro Arg Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
145 150 155 160

caa aat ggc gtc c
493
Gln Asn Gly Val

<210> 42
<211> 164
<212> PRT
<213> Mus musculus

<220>
<221> VARIANT
<222> 2
<223> Xaa is Val

<221> VARIANT
<222> 3
<223> Xaa is Phe or Leu

<221> VARIANT
<222> 5
<223> Xaa is Thr

<221> VARIANT
<222> 7
<223> Xaa is Thr

<400> 42
Asp Xaa Xaa Met Xaa Gln Xaa Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser
20 25 30
Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly
85 90 95
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
115 120 125
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
130 135 140
Tyr Pro Arg Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
145 150 155 160
Gln Asn Gly Val

<210> 43
<211> 354
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(354)
<223> partial nucleotide sequence encoding B436 heavy
(IgG2a) chain

<400> 43
gag gty atg ytk gty gar tct gga gga ggc tta gtg aag cct gga ggg
48
Glu Xaa Met Xaa Xaa Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

tcc ctg aaa ctc tcc tgt gta gcc tct gga ttc act ttc agt aga tat
96
Ser Leu Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

acc atg tct tgg gtt cgc cag act ccg gcg aag aga ctg gag tgg gtc
144
Thr Met Ser Trp Val Arg Gln Thr Pro Ala Lys Arg Leu Glu Trp Val
35 40 45

gca acc atc aat ttt ggt aat ggt aac acc tac tat cct gac agt gtg
192
Ala Thr Ile Asn Phe Gly Asn Gly Asn Thr Tyr Tyr Pro Asp Ser Val
50 55 60

aag ggc cga ttc acc atc tcc aga gac aat gcc agg aac acc ctg tat
240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Thr Leu Tyr
65 70 75 80

ctg caa atg agc agt ctg agg tct gag gac acg gcc atg tat tac tgt
288
Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

aca agc ctt aat tgg gct tac tgg ggc caa ggg act ctg gtc acc gtc
336
Thr Ser Leu Asn Trp Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
100 105 110

tcc tca gcc aaa aca aca
354
Ser Ser Ala Lys Thr Thr
115

<210> 44
<211> 118
<212> PRT
<213> Mus musculus

<220>
<221> VARIANT
<222> 2
<223> Xaa is Val

<221> VARIANT
<222> 4
<223> Xaa is Phe or Leu

<221> VARIANT
<222> 5
<223> Xaa is Val

<400> 44
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Glu Xaa Met Xaa Xaa Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1               5                  10                  15
Ser Leu Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Arg Tyr
            20                  25                  30
Thr Met Ser Trp Val Arg Gln Thr Pro Ala Lys Arg Leu Glu Trp Val
        35                  40                  45
Ala Thr Ile Asn Phe Gly Asn Gly Asn Thr Tyr Tyr Pro Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95
Thr Ser Leu Asn Trp Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
            100                 105                 110
Ser Ser Ala Lys Thr Thr
        115
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<210> 45
<211> 36
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(36)
<223> Mouse immunoglobulin Light Chain J segment

<300>
<308> GenBank #z66550
<309> 1997-12-15

<400> 45

tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa
36
Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 46
<211> 12
<212> PRT
<213> Mus musculus

<400> 46
Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 47
<211> 21
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(21)
<223> Mouse immunoglobulin Light Chain V-J region

<300>
<308> GenBank #Z66550
<309> 1997-12-15

<400> 47
cta cag tat gat gag ctt cca
21
Leu Gln Tyr Asp Glu Leu Pro
1 5

<210> 48
<211> 7
<212> PRT
<213> Mus musculus

<400> 48.
Leu Gln Tyr Asp Glu Leu Pro
1 5

<210> 49
<211> 34
<212> DNA
<213> Mus musculus

<220>
<221> CDS

<222> (1)...(34)
<223> Mouse Immunoglobulin Light Chain J region

<300>
<308> GenBank #Z31353
<309> 1995-09-27

<400> 49
gac gtt cgg tgg agg cac caa gct gga aat caa a
34
Asp Val Arg Trp Arg His Gln Ala Gly Asn Gln
 1   5   10

<210> 50
<211> 11
<212> PRT
<213> Mus musculus

<400> 50
Asp Val Arg Trp Arg His Gln Ala Gly Asn Gln
 1   5   10

<210> 51
<211> 39
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(39)
<223> Mouse Immunoglobulin Light Chain J region

<300>
<308> GenBank #X87231
<309> 1995-06-06

<400> 51
gtg gac gtt cgg tgg agg cac caa gct gga aat caa acg
39
Val Asp Val Arg Trp Arg His Gln Ala Gly Asn Gln Thr
 1   5   10

<210> 52
<211> 13
<212> PRT
<213> Mus musculus

<400> 52
Val Asp Val Arg Trp Arg His Gln Ala Gly Asn Gln Thr
 1   5   10

<210> 53
<211> 33
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(33)
<223> mouse immunoglobulin light chain J region

<300>
<308> GenBank #L21025
<309> 1994-10-29

<400> 53
tgg acg ttc ggt gga ggc acc aag ctg gaa atc
33
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
1 5 10

<210> 54
<211> 11
<212> PRT
<213> Mus musculus

<400> 54
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
1 5 10

<210> 55
<211> 12
<212> PRT
<213> Mus musculus

<220>
<221> DOMAIN
<222> (1)...(12)
<223> Mouse immunoglobulin light chain J-region

<300>
<308> GenBank #Z6650
<309> 1997-12-15

<400> 55
Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 56
<211> 36
<212> DNA

<213> Mus musculus

<220>
<221> CDS
<222> (1)...(36)
<223> Mouse immunoglobulin light chain J region

<300>
<308> GenBank #L21021
<309> 1994-10-29

<400> 56
ctg acg ttc ggt gga ggc acc aag ctg gaa atc aat
36
Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn
1 5 10

<210> 57
<211> 12
<212> PRT
<213> Mus musculus

<400> 57
Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn
1 5 10

<210> 58
<211> 36
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(36)
<223> mouse immunoglobulin light chain J region

<300>
<308> GenBank #L21019
<309> 1994-10-29

<400> 58
ccg acg ttc ggt gga ggc acc aag ctg gaa atc acc
36
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
1 5 10

<210> 59
<211> 12
<212> PRT
<213> Mus musculus

<400> 59
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
1 5 10

<210> 60
<211> 36
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(36)
<223> mouse immunoglobulin light chain J region

<300>
<308> GenBank #L21017
<309> 1994-10-29

<400> 60
ccg acg ttc ggt gga ggc acc aag ctg gaa atc aaa
36
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 61
<211> 12
<212> PRT
<213> Mus musculus

<400> 61
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 62
<211> 319
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(319)
<223> mouse immunoglobulin light chain Constant region

<300>
<308> GenBank #X87231
<309> 1995-06-06

<400> 62

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ggc tga tgc tgc acc aac tgt atc cat ctt ccc acc atc cag tga gca
48
Gly     Cys Cys Thr Asn Cys Ile His Leu Pro Thr Ile Gln     Ala
 1                  5                   10

gtt aac atc tgg agg tgc ctc agt cgt gtg ctt ctt gaa caa ctt cta
96
Val Asn Ile Trp Arg Cys Leu Ser Arg Val Leu Leu Glu Gln Leu Leu
 15                 20                  25                   30

ccc caa aga cat caa tgt caa gtg gaa gat tga tgg cag tga acg aca
144
Pro Gln Arg His Gln Cys Gln Val Glu Asp     Trp Gln     Thr Thr
                35                  40

aaa tgg cgt cct gaa cag ttg gac tga tca gga cag caa aga cag cac
192
Lys Trp Arg Pro Glu Gln Leu Asp     Ser Gly Gln Gln Arg Gln His
 45                 50                      55

cta cag cat gag cag cac cct cac gtt gac caa gga cga gta tga acg
240
Leu Gln His Glu Gln His Pro His Val Asp Gln Gly Arg Val     Thr
 60                 65                  70

aca taa cag cta tac ctg tga ggc cac tca caa gac atc tac ttc acc
288
Thr     Gln Leu Tyr Leu     Gly His Ser Gln Asp Ile Tyr Phe Thr
 75                         80                  85

cat tgt caa gag ctt caa cag gaa tga gtg t
319
His Cys Gln Glu Leu Gln Gln Glu     Val
     90                 95


<210> 63
<211> 97
<212> PRT
<213> Mus musculus

<400> 63
Gly Cys Cys Thr Asn Cys Ile His Leu Pro Thr Ile Gln Ala Val Asn
 1              5                   10                  15
Ile Trp Arg Cys Leu Ser Arg Val Leu Leu Glu Gln Leu Leu Pro Gln
            20                  25                  30
Arg His Gln Cys Gln Val Glu Asp Trp Gln Thr Thr Lys Trp Arg Pro
        35                  40                  45
Glu Gln Leu Asp Ser Gly Gln Gln Arg Gln His Leu Gln His Glu Gln
    50                  55                  60
His Pro His Val Asp Gln Gly Arg Val Thr Thr Gln Leu Tyr Leu Gly
65                  70                  75                  80
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His Ser Gln Asp Ile Tyr Phe Thr His Cys Gln Glu Leu Gln Gln Glu
                85   90   95
Val

<210> 64
<211> 321
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(321)
<223> mouse partial nucleotide sequence for
      immunoglobulin light chain Constant region

<300>
<308> GenBank #AJ294736
<309> 2001-02-09

<400> 64
gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag cag
48
Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
 1   5   10   15

tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc tac
96
Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
            20   25   30

ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga caa
144
Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
        35   40   45

aat ggc gtc ctg aac agt tgg act gat cag gac agc aaa gac agc acc
192
Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
    50   55   60

tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa cga
240
Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
65   70   75   80

cat aac agc tat acc tgt gag gcc act cac aag aca tca act tca ccc
288
His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
                85   90   95

atc gtc aag agc ttc aac agg aat gag tgt tag
321
Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
100 105

<210> 65
<211> 106
<212> PRT
<213> Mus musculus

<400> 65
Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
1 5 10 15
Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
20 25 30
Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
35 40 45
Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
50 55 60
Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
65 70 75 80
His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
85 90 95
Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
100 105

<210> 66
<211> 48
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(48)
<223> mouse antibody heavy chain J region

<300>
<308> GenBank #Z66553
<309> 1997-12-15

<400> 66
tgg aac ttc gat gtc tgg ggc gca ggg acc acg gtc acc gtc tcc tca
48
Trp Asn Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
1 5 10 15

<210> 67
<211> 15
<212> PRT
<213> Mus musculus

<400> 67
Asn Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
1 5 10 15

<210> 68
<211> 990
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(991)
<223> mouse immunoglobulin heavy chain Constant region

<300>
<308> GenBank #AJ294738
<309> 2001-02-09

<400> 68
gcc aaa aca aca gcc cca tcg gtc tat cca ctg gcc cct gtg tgt gga
48
Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly
1 5 10 15

gat aca act ggc tcc tcg gtg act cta gga tgc ctg gtc aag ggt tat
96
Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
20 25 30

ttc cct gag cca gtg acc ttg acc tgg aac tct gga tcc ctg tcc agt
144
Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser
35 40 45

ggt gtg cac acc ttc cca gct gtc ctg cag tct gac ctc tac acc ctc
192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
50 55 60

agc agc tca gtg act gta acc tcg agc acc tgg ccc agc cag tcc atc
240
Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
65 70 75 80

acc tgc aat gtg gcc cac ccg gca agc agc acc aag gtg gac aag aaa
288
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
85 90 95

att gag ccc aga ggg ccc aca atc aag ccc tgt cct cca tgc aaa tgc
336

Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
100 105 110

cca gca cct aac ctc ttg ggt gga cca tcc gtc ttc atc ttc cct cca
384
Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
115 120 125

aag atc aag gat gta ctc atg atc tcc ctg agc ccc ata gtc aca tgt
432
Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
130 135 140

gtg gtg gtg gat gtg agc gag gat gac cca gat gtc cag atc agc tgg
480
Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
145 150 155 160

ttt gtg aac aac gtg gaa gta cac aca gct cag aca caa acc cat aga
528
Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
165 170 175

gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc cag
576
Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
180 185 190

cac cag gac tgg atg agt ggc aag gag ttc aaa tgc aag gtc aac aac
624
His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
195 200 205

aaa gac ctc cca gcg ccc atc gag aga acc atc tca aaa ccc aaa ggg
672
Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly
210 215 220

tca gta aga gct cca cag gta tat gtc ttg cct cca cca gaa gaa gag
720
Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu
225 230 235 240

atg act aag aaa cag gtc act ctg acc tgc atg gtc aca gac ttc atg
768
Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
245 250 255

cct gaa gac att tac gtg gag tgg acc aac aac ggg aaa aca gag cta
816
Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
260 265 270

aac tac aag aac act gaa cca gtc ctg gac tct gat ggt tct tac ttc 864
Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe
275 280 285

atg tac agc aag ctg aga gtg gaa aag aag aac tgg gtg gaa aga aat 912
Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
290 295 300

agc tac tcc tgt tca gtg gtc cac gag ggt ctg cac aat cac cac acg 960
Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr
305 310 315 320

act aag agc ttc tcc cgg act ccg ggt aaa 990
Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
325 330

<210> 69
<211> 329
<212> PRT
<213> Mus musculus

<400> 69
Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp
1 5 10 15
Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe
20 25 30
Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly
35 40 45
Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser
50 55 60
Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr
65 70 75 80
Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile
85 90 95
Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro
100 105 110
Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
115 120 125
Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val
130 135 140
Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe
145 150 155 160
Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu
165 170 175
Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His
180 185 190

Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys
195 200 205
Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser
210 215 220
Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met
225 230 235 240
Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro
245 250 255
Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn
260 265 270
Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met
275 280 285
Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser
290 295 300
Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr
305 310 315 320
Lys Ser Phe Ser Arg Thr Pro Gly Lys
325

<210> 70
<211> 975
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(972)
<223> mouse immunoglobulin heavy chain Constant region

<300>
<308> GenBank #M60435
<309> 2001-05-16

<400> 70
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48
Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala
1 5 10 15

gcc caa act aac tcc atg gtg acc ctg gga tgc ctg gtc aag ggc tat
96
Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
20 25 30

ttc cct gag cca gtg aca gtg acc tgg aac tct gga tcc ctg tcc agc
144
Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser
35 40 45

ggt gtg cac acc ttc cca gct gtc ctg cag tct gac ctc tac act ctg
192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu

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        50                  55                  60

agc agc tca gtg act gtc ccc tcc agc acc tgg ccc agc gag acc gtc
240
Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val
 65                 70                  75                  80

acc tgc aac gtt gcc cac ccg gcc agc agc acc aag gtg gac aag aaa
288
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
                85                  90                  95

att gtg ccc agg gat tgt ggt tgt aag cct tgc ata tgt aca gtc cca
336
Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
            100                 105                 110

gaa gta tca tct gtc ttc atc ttc ccc cca aag ccc aag gat gtg ctc
384
Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
        115                 120                 125

acc att act ctg act cct aag gtc acg tgt gtt gtg gta gac atc agc
432
Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser
    130                 135                 140

aag gat gat ccc gag gtc cag ttc agc tgg ttt gta gat gat gtg gag
480
Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
145                 150                 155                 160

gtg cac aca gct cag acg caa ccc cgg gag gag cag ttc aac agc act
528
Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr
                165                 170                 175

ttc cgc tca gtc agt gaa ctt ccc atc atg cac cag gac tgg ctc aat
576
Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
            180                 185                 190

ggc aag gag ttc aaa tgc agg gtc aac agt gca gct ttc cct gcc ccc
624
Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
        195                 200                 205

atc gag aaa acc atc tcc aaa acc aaa ggc aga ccg aag gct cca cag
672
Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
    210                 215                 220
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gtg tac acc att cca cct ccc aag gag cag atg gcc aag gat aaa gtc
720
Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
225 230 235 240

agt ctg acc tgc atg ata aca gac ttc ttc cct gaa gac att act gtg
768
Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
245 250 255

gag tgg cag tgg aat ggg cag cca gcg gag aac tac aag aac act cag
816
Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
260 265 270

ccc atc atg gac aca gat ggc tct tac ttc gtc tac agc aag ctc aat
864
Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
275 280 285

gtg cag aag agc aac tgg gag gca gga aat act ttc acc tgc tct gtg
912
Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
290 295 300

tta cat gag ggc ctg cac aac cac cat act gag aag agc ctc tcc cac
960
Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
305 310 315 320

tct cct ggt aaa tga
975
Ser Pro Gly Lys

<210> 71
<211> 324
<212> PRT
<213> Mus musculus

<400> 71
Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala
1 5 10 15
Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
20 25 30
Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser
35 40 45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
50 55 60
Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val
65 70 75 80

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Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
                85                  90                  95
Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
            100                 105                 110
Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
        115                 120                 125
Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser
    130                 135                 140
Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
145                 150                 155                 160
Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr
                165                 170                 175
Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
            180                 185                 190
Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
        195                 200                 205
Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
    210                 215                 220
Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
225                 230                 235                 240
Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
                245                 250                 255
Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
            260                 265                 270
Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
        275                 280                 285
Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
    290                 295                 300
Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
305                 310                 315                 320
Ser Pro Gly Lys
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<210> 72
<211> 41
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(41)
<223> Human immunoglobulin light chain J-region

<300>
<308> GenBank #D90159
<309> 2002-05-29

<400> 72

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ctc act ttc ggc gga ggg acc aag gtg gag atc aaa cgt aa
41
Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
 1               5                  10
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<210> 73
<211> 13
<212> PRT
<213> Homo sapien

<400> 73
Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

<210> 74
<211> 37
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(37)
<223> Human immunoglobulin kappa light chain J region

<300>
<308> GenBank #D28523
<309> 2002-03-26

<400> 74
ctt tcg gcc ctg gga cca aag tgg ata tca aac gta a
37
Leu Ser Ala Leu Gly Pro Lys Trp Ile Ser Asn Val
1 5 10

<210> 75
<211> 12
<212> PRT
<213> Homo sapiens

<400> 75
Leu Ser Ala Leu Gly Pro Lys Trp Ile Ser Asn Val
1 5 10

<210> 76
<211> 37
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(37)
<223> Human immunoglobulin kappa light chain J-region

<300>

<308> GenBank #Z00020
<309> 1997-02-17

<400> 76
tgg acg ttc ggc caa ggg acc aag gtg gaa atc aaa c
37
Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1 5 10

<210> 77
<211> 12
<212> PRT
<213> Homo sapiens

<400> 77
Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1 5 10

<210> 78
<211> 34
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(34)
<223> Human immunoglobulin light chain J segment

<300>
<308> GenBank #X72747
<309> 1994-01-28

<400> 78
gta ttc ggc gga ggg acc aag ctg acc gtc cta g
34
Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
1 5 10

<210> 79
<211> 11
<212> PRT
<213> Homo sapiens

<400> 79
Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
1 5 10

<210> 80
<211> 318
<212> DNA

<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(318)
<223> Human immunoglobulin light chain Constant region

<300>
<308> GenBank #AF113889
<309> 2000-01-28

<400> 80
act gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag
48
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1   5   10   15

ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat
96
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
            20   25   30

ccc aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg
144
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
        35   40   45

ggt aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc
192
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
    50   55   60

tac agc ctc agc aac acc ctg acg ctg agc aaa gca gac tac gag aaa
240
Tyr Ser Leu Ser Asn Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 65   70   75   80

cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc
288
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
                85   90   95

gtc aca aag agc ttc aac agg gga gag tgc
318
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100   105

<210> 81
<211> 106
<212> PRT
<213> Homo sapiens

```
<400> 81
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1               5                  10                  15
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
            20                  25                  30
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
        35                  40                  45
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
    50                  55                  60
Tyr Ser Leu Ser Asn Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65                  70                  75                  80
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
                85                  90                  95
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105

<210> 82
<211> 981
<212> DNA
<213> Homo Sapien

<220>
<221> CDS
<222> (1)...(326)
<223> Immunoglobulin heavy chain constant region

<300>
<308> GenBank #AJ250170
<309> 2001-11-22

<400> 82
gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gcg ccc tgc tcc agg    48
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1               5                  10                  15

agc acc tcc gag agc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac    96
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gct ctg acc agc   144
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc   192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

ctc agc agc gtg gtg acc gtg acc tcc agc aac ttc ggc acc cag acc   240
Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80
```

```
tac acc tgc aac gta gat cac aag ccc agc aac acc aag gtg gac aag     288
Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

aca gtt gag cgc aaa tgt tgt gtc gag tgc cca ccg tg cccagcacca      336
Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
            100                 105

cctgtggcag gaccgtcagt cttcctcttc cccccaaaac ccaaggacac cctcatgatc 396
tcccggaccc ctgaggtcac gtgcgtggtg gtggacgtga gccacgaaga ccccgaggtc 456
cagttcaact ggtacgtgga cggcatggag gtgcataatg ccaagacaaa gccacgggag 516
gagcagttca acagcacgtt ccgtgtggtc agcgtcctca ccgtcgtgca ccaggactgg 576
ctgaacggca aggagtacaa gtgcaaggtc tccaacaaag gcctcccagc ccccatcgag 636
aaaaccatct ccaaaaccaa agggcagccc cgagaaccac aggtgtacac cctgccccca 696
tcccgggagg agatgaccaa gaaccaggtc agcctgacct gcctggtcaa aggcttctac 756
cccagcgaca tcgccgtgga gtgggagagc aatgggcagc cggagaacaa ctacaagacc 816
acacctccca tgctggactc cgacggctcc ttcttcctct acagcaagct caccgtggac 876
aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg tgatgcatga ggctctgcac 936
aaccactaca cacagaagag cctctccctg tctccgggta aatga                 981
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<210> 83
<211> 108
<212> PRT
<213> Homo Sapien

<400> 83

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Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1               5                  10                  15
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60
Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80
Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95
Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
            100                 105
```

<210> 84
<211> 654
<212> DNA
<213> Homo Sapien

<220>
<221> CDS
<222> (1)...(217)
<223>Immunoglobulin heavy chain constant region

<300>
<308> GenBank #AJ001564
<309> 1999-10-08

<400> 84
gca cct gag ttc ctg ggg gga cca tca gtc ttc ctg ttc ccc cca aaa 48
Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1 5 10 15

ccc aag gac act ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg 96
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30

gtg gtg gac gtg agc cag gaa gac ccc gag gtc cag ttc aac tgg tac 144
Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
35 40 45

gtg gat ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag 192
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60

cag ttc aac agc acg tac cgt gtg g tcagcgtcct caccgtcctg 237
Gln Phe Asn Ser Thr Tyr Arg Val
65 70

caccaggact ggctgaacgg caaggagtac aagtgcaagg tctccaacaa aggcctcccg 297
tcctccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagagcc acaggtgtac 357
accctgcccc catcccagga ggagatgacc aagaaccagg tcagcctgac ctgcctggtc 417
aaaggcttct accccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac 477
aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttcct ctacagcaag 537
ctcaccgtgg acaagagcag gtggcaggag gggaacgtct tctcatgctc cgtgatgcat 597
gaggctctgc acaaccacta cacgcagaag agcctctccc tgtctctggg taaatga 654

<210> 85
<211> 72
<212> PRT
<213> Homo Sapien

<400> 85
Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1 5 10 15
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30
Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
35 40 45
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60
Gln Phe Asn Ser Thr Tyr Arg Val
65 70

<210> 86

<211> 690
<212> DNA
<213> Homo Sapien

<220>
<221> CDS
<222> (1)...(229)
<223>Immunoglobulin heavy chain constant region

<300>
<308> GenBank #AJ001563
<309> 1999-10-08

<400> 86
gag tcc aaa tat ggt ccc ccg tgc cca tca tgc cca gca cct gag ttc 48
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
1 5 10 15

ctg ggg gga cca tca gtc ttc ctg ttc ccc cca aaa ccc aag gac act 96
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
20 25 30

ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg 144
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
35 40 45

agc cag gaa gac ccc gag gtc cag ttc aac tgg tac gtg gat ggc gtg 192
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
50 55 60

gag gtg cat aat gcc aag aca aag ccg cgg gag gag c agttcaacag 239
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
65 70 75

cacgtaccgt gtggtcagcg tcctcaccgt cgtgcaccag gactggctga acggcaagga 299
gtacaagtgc aaggtctcca acaaaggcct cccgtcctcc atcgagaaaa ccatctccaa 359
agccaaaggg cagccccgag agccacaggt gtacaccctg cccccatccc aggaggagat 419
gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc ttctacccca gcgacatcgc 479
cgtggagtgg gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct 539
ggactccgac ggctccttct tcctctacag caggctaacc gtggacaaga gcaggtggca 599
ggaggggaat gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca 659
gaagagcctc tccctgtctc tgggtaaatg a 690

<210> 87
<211> 76
<212> PRT
<213> Homo Sapien

<400> 87
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
1 5 10 15
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr

```
                20                  25                  30
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            35                  40                  45
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
        50                  55                  60
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
65                  70                  75
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<210> 88
<211> 60
<212> DNA
<213> Homo Sapien

<220>
<221> CDS
<222> (1)...(60)
<223>Immunoglobulin heavy chain J region

<300>
<308> GenBank #M63030
<309> 1995-01-03

<400> 88

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tta cta cta cta cta cta cat gga cgt ctg ggg caa agg gac cac ggt      48
Leu Leu Leu Leu Leu Leu His Gly Arg Leu Gly Gln Arg Asp His Gly
 1               5                  10                  15

cac cgt ctc ctc                                                      60
His Arg Leu Leu
            20
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<210> 89
<211> 20
<212> PRT
<213> Homo Sapien

<400> 89

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Leu Leu Leu Leu Leu Leu His Gly Arg Leu Gly Gln Arg Asp His Gly
 1               5                  10                  15
His Arg Leu Leu
            20
```

<210> 90
<211> 60
<212> DNA
<213> Homo Sapien

<220>
<221> CDS
<222> (1)...(60)
<223>Immunoglobulin heavy chain J region

<300>
<308> GenBank #M63031
<309> 1995-01-03

<400> 90
tta cta cta cta cta cgg tat gga cgt ctg ggg cca agg gac cac ggt 48
Leu Leu Leu Leu Leu Arg Tyr Gly Arg Leu Gly Pro Arg Asp His Gly
1 5 10 15

cac cgt ctc ctc 60
His Arg Leu Leu
20

ccgtctcctc 60

<210> 91
<211> 20
<212> PRT
<213> Homo Sapien

<400> 91
Leu Leu Leu Leu Leu Arg Tyr Gly Arg Leu Gly Pro Arg Asp His Gly
1 5 10 15
His Arg Leu Leu
20

<210> 92
<211> 6
<212> PRT
<213> Homo Sapien

<400> 92
Leu Leu Leu Leu Leu Arg
1 5

<210> 93
<211> 20
<212> PRT
<213> Homo Sapien

<400> 93
Leu Leu Leu Leu Leu Arg Tyr Gly Arg Leu Gly Pro Arg Asp His Gly
1 5 10 15
His Arg Leu Leu
20

<210> 94
<211> 60

<212> DNA
<213> Homo Sapien

<220>
<221> CDS
<222> (1)...(20)

<400> 94
tta cta cta cta cta cta ca tggacgtctg gggcaaaggg accacggtca 50
Leu Leu Leu Leu Leu Leu
 1 5

ccgtctcctc 60

<210> 95
<211> 6
<212> PRT
<213> Homo Sapien

<400> 95
Leu Leu Leu Leu Leu Leu
 1 5

<210> 96
<211> 20
<212> PRT
<213> Homo Sapien

<400> 96
Leu Leu Leu Leu Leu Leu His Gly Arg Leu Gly Gln Arg Asp His Gly
 1 5 10 15
His Arg Leu Leu
 20

<210> 97
<211> 214
<212> PRT
<213> Mus musculus

<400> 97
Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
 1 5 10 15
Asp Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
 20 25 30
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Ile Leu Ile
 35 40 45
Lys Tyr Ala Ser Gln Ser Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
Ser Gly Ser Gly Thr Phe Phe Thr Leu Ile Val Asn Ser Val Gly Thr
65 70 75 80

Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser His Ser Trp Pro Leu
85 90 95
Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala
100 105 110
Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115 120 125
Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Arg Asp Ile
130 135 140
Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
145 150 155 160
Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
165 170 175
Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr
180 185 190
Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
195 200 205
Phe Asn Arg Asn Glu Cys
210

<210> 98
<211> 442
<212> PRT
<213> Mus musculus

<400> 98
Glu Val Lys Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ala Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Asp
20 25 30
Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45
Ala Ser Ile Ser Ser Val Gly Asn Thr Tyr Tyr Pro Asp Ser Val Lys
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
65 70 75 80
Gln Met Ser Ser Val Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95
Arg Gly Tyr Gly Val Ser Pro Trp Phe Ser Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro
115 120 125
Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly
130 135 140
Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn
145 150 155 160
Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr
180 185 190
Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser
195 200 205

```
Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro
    210                 215                 220
Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro
225                 230                 235                 240
Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys
                245                 250                 255
Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp
            260                 265                 270
Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu
        275                 280                 285
Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met
    290                 295                 300
His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser
305                 310                 315                 320
Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
                325                 330                 335
Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln
            340                 345                 350
Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe
        355                 360                 365
Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu
    370                 375                 380
Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe
385                 390                 395                 400
Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn
                405                 410                 415
Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr
            420                 425                 430
Glu Lys Ser Leu Ser His Ser Pro Gly Lys
        435                 440


<210> 99
<211> 219
<212> PRT
<213> Mus musculus

<400> 99
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1               5                  10                  15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30
Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly
                85                  90                  95
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
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Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
115 120 125
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
130 135 140
Tyr Pro Arg Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
145 150 155 160
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
165 170 175
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
180 185 190
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
195 200 205
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
210 215

<210> 100
<211> 118
<212> PRT
<213> Mus musculus

<400> 100
Glu Val Met Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30
Thr Met Ser Trp Val Arg Gln Thr Pro Ala Lys Arg Leu Glu Trp Val
35 40 45
Ala Thr Ile Asn Phe Gly Asn Gly Asn Thr Tyr Tyr Pro Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Thr Ser Leu Asn Trp Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
100 105 110
Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly
115 120 125
Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys
130 135 140
Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu
145 150 155 160
Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr
165 170 175
Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu
180 185 190
Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp
195 200 205
Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr
210 215 220
Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp
225 230 235 240

Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp
245 250 255
Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp
260 265 270
Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn
275 280 285
Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp
290 295 300
Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro
305 310 315 320
Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala
325 330 335
Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp
340 345 350
Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile
355 360 365
Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn
370 375 380
Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys
385 390 395 400
Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys
405 410 415
Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu
420 425 430
Ser His Ser Pro Gly Lys
435